Inquiry-Based Biotechnology Education

For Kent Intermediate School District Early College Program

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Dedication Page

This thesis is dedicated to my loving husband, Nick, who never doubted my ability in the same way I sometimes did, and to my dear stepson, Matthew, for his understanding of our missed time together.

"Tell me and I forget, show me and I remember, involve me and I understand." - Chinese Proverb

"It is nothing short of a miracle that the modern methods of instruction have not yet entirely strangled the holy curiosity of enquiry." - Albert Einstein, Ideas and Opinions

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Abstract

Growth in the field of biotechnology, combined with the ability to access information instantaneously, requires a new model of science education that will nurture deeper understanding and higher order thinking to develop a scientifically literate population. Inquiry-based learning is a student-centered model built on the theoretical framework of constructivism, which allows students to learn in a way that reflects how scientists come to understand the natural world. This project aimed to address the need for an inquiry-based biotechnology curriculum in a local Early College program by developing, piloting, revising, and implementing an inquiry-based biotechnology unit while simultaneously evaluating the impact of this curriculum on content knowledge and students' motivation toward science learning. Results revealed that student assignment scores were consistent with a B- average and performance on the final presentation was consistent with an A- average, while content knowledge increased approximately 9 to 19 percentage points comparing pretest and posttest. Overall, using the Student Motivation Toward Science Learning survey, we did not see any measurable changes in students' motivation toward science learning except for a slight decrease in self-efficacy, which could be reasonably expected given student discomfort experiencing both a novel curriculum and pedagogy. Qualitative student feedback, however, was positive regarding independence, accountability, and group discussion and students displayed a high level of enjoyment with the hands-on activities. Thus, this project resulted in a sample inquiry-based biotechnology curriculum unit that produced reasonable gains in content knowledge, and with further work on the affective components important to cognitive growth, displays potential for even larger content knowledge gains and increased student motivation toward science learning.

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Chapter I – Introduction and Background

Problem and Rationale

With the advent of the Human Genome Project, stem cell research, advances in gene therapy, and the use of genetically modified organisms biotechnology has become integrated into decisions ranging from a personal to a political level (Hengan and Bigler, 2009), thus driving a need for a population possessing scientific literacy in this area. However, developing scientific literacy in biotechnology requires more than the traditional dissemination of facts, as the ability to memorize information has become less important, while the ability to make sense of that information and apply it has become more important (Gilbert, 2005).

The prevalent model for science education throughout the twentieth century was one that emphasized the dissemination of knowledge from teacher to learner. Although this teacher-centered approach was accepted during that era, not everyone supported it, and the changing needs of our society now demand a new model (Friesen, 2009). In previous decades, memorization of facts was important and had its place. However, we now live in a society where the ability to access information is nearly instantaneous and the volume of information is growing exponentially.

This paradigm shift requires a new model of science education, one that will nurture a deeper understanding of science and higher order thinking regarding its related topics, particularly those in the area of biotechnology. Inquiry-based learning is a student-centered model built on the theoretical framework of constructivism, whereby the learner generates knowledge through the interaction between experiences and ideas (Duffy & Cunningham, 1996). Inquiry allows students to learn in a way that reflects how scientists come to understand the natural world. This method of teaching and learning has actually been in use since the time of

Socrates and has had various supporters throughout history such as Jean Piaget, Jerome Bruner, Lev Vygotsky, and John Dewey (Piaget, 1964; Bruner, 1961; Vygotsky, 1978; Dewey, 1910).

More recently, inquiry has been shown to be aligned with best practices in the teaching and learning of science. Inquiry is at the foundation of the *Next Generation Science Standards* (NGSS) (Lead States, 2013), and the cognitive, social, and physical practices required by inquiry are extensively articulated within Dimension I of *A Framework for K-12 Science Education* (NRC, 2012). Inquiry-based instruction has been shown to improve student learning and short term retention of science content, as well as student competencies and confidence in their ability to practice science (Singer, Nielsen, & Schweingruber, 2012). It also has the potential for developing a citizenry that possess the skills to both make sense of scientific information and apply this information in order to solve critical problems (Bigler & Hanegan, 2011; DeHaan, 2005; National Research Council, 1996), yet it has not been widely adopted across the discipline (Bigler & Hanegan, 2011).

Somewhat similarly, despite the significance of biotechnology within the sciences and its applications within society today, it too has not been widely adopted within the science curriculum (Borgerding, Sadler, & Koroly, 2013). Educators cite various challenges to implementing both inquiry-based instruction and biotechnology education in their classrooms including accessibility of investigation techniques and management of extended activities, and comfort with the content knowledge and equipment (Borgerding, Sadler, & Koroly, 2013; Edelson, Gordin, & Pea, 1999).

Background

The original impetus for developing a biotechnology curriculum was the request for one from a local Early College educator. As is common with Early College programs, one goal is for

the students involved to earn dual credit, or credit that will count both for their secondary requirements and be recognized by partnering institutions of higher education. By introducing biotechnology to the current curriculum, the local Early College program could continue to expand on the goals for its students in the area of credit articulation.

Biotechnology is a subfield in the area of life sciences, and is increasingly being used in the areas of agriculture, industry, and medicine (Steele & Aubusson, 2004). Merriam-Webster defines biotechnology as "the manipulation of living organisms for their components to produce useful usually commercial products" (Biotechnology, 2015). Other definitions are broader and include concepts such as the use of recombinant DNA techniques to modify life forms (Plein, 1991). As such, biotechnology is a perfect marriage between content and process, providing an opportunity for the interactive experience that educators and researchers support as being the heart of learning science and in alignment with inquiry pedagogy (Hanegan and Bigler, 2009).

Biotechnology education is important because today's students will be responsible for making decisions regarding the development and use of these technologies in the short future (Steele & Aubusson, 2004). Given this increasing importance, Hanegan and Bigler (2009) would expect to see its integration at all levels of science education. However, although a National Science Foundation funded survey of high school biology teachers found that hands-on biotechnology education is available through advanced biology courses; similar experiences were non-existent in mainstream biology coursework in the United States (Hanegan and Bigler, 2009). Even where biotechnology education was being implemented, the focus was primarily on content (96%) rather than process (4%) (Mansius & Hanegan, 2008). Furthermore, a preliminary study in Utah, which surveyed 42 biology teachers from across the state about their biotechnology education practices, found that not a single educator was incorporating inquiry

into their lessons (Mansius & Hanegan, 2008) and one may infer other states may share in a similar diagnosis. When asked why biotechnology educators do not incorporate inquiry into their teaching practices, many respond they do not have the time to teach with inquiry (Hanegan and Bigler, 2009), and therefore, resort to traditional lecture and verification laboratory experiments. Ketpichainarong et al. (2010) suggest an obstacle may be the lack of preparedness for inquiry on the part of the instructor, while Bigler and Hanegan (2011) also identified a lack of knowledge with biotechnology equipment as a hurdle.

Biotechnology curriculum is limited at the K-12 level. This is perhaps why there is also little research in the area of inquiry teaching in biotechnology at the K-12 level. The research that does exist regarding inquiry teaching at the K-12 level most often addresses this form of pedagogy in general science courses (Ergül et al., 2011; Gengarelly & Abrams, 2009). The research which addresses inquiry learning in the specific discipline of biotechnology comes from studies of this approach implemented at the undergraduate postsecondary level (Friedel et al., 2008; Ketpichainarong et al., 2010; Lesmes Celorrio et al., 2013). Given the current state of biotechnology education and educators' reservations, developing a high school level inquiry-based biotechnology unit is ideal for filling a current need in practice within the field. Introducing it at the early college level is a natural transition in preparing secondary students for the sort of curriculum they are likely to encounter in their post-secondary science education experience (Buck, Bretz, & Towns, 2008; Ketpichainarong, Panijpan, & Ruenwongsa, 2010; Roth, McGinn, & Bowen, 1998).

Purpose of the Study

The focus of this study is on the unique intersection of biotechnology education and inquiry-based pedagogy. The purpose of this study is two-fold. The first purpose is to address the

need for a hands-on, inquiry-based curriculum that will introduce biotechnology into the teaching of introductory and foundational biology concepts to meet the specific needs of a local Early College program. Second, this study aims to evaluate the impact of this inquiry-based biotechnology curriculum on student learning outcomes in the area of content knowledge and students' motivation toward science learning using pre and posttests, pre- and post-assessments, qualitative observations, and student feedback. Because there is a lack of adoption of both inquiry-based teaching and learning (Crawford, 2007; Zion & Mendelovici, 2012) and biotechnology education (Bigler & Hanegan, 2011; Borgerding, Sadler, & Koroly, 2013) within the science curriculum, this study aims to add to the literature and fill this gap in the curriculum by providing a case study of inquiry-based biotechnology education.

Guiding Research Questions

The objective of this study is to meet the need of a local Early College program while simultaneously filling a gap within the broader field and literature. As such, the research questions are:

1. What are the effects of an inquiry-based biotechnology unit on students' content knowledge?
2. How do students' motivation toward science learning differ between pre- and post-assessment after engaging in an inquiry-based biotechnology unit?

Because there is further room for adoption of both inquiry-based pedagogy (Crawford, 2007; Zion & Mendelovici, 2012) and biotechnology education (Bigler & Hanegan, 2011; Borgerding, Sadler, & Koroly, 2013) within the science curriculum, this exploratory case study will add to both the literature and curricula in this area. Due to the design of the study, there were no formal hypotheses concerning the outcomes. It was expected, however, that student learning

outcome scores would increase between pre- and post-assessment and that student perceptions regarding their learning through inquiry would be more positive on the post-assessment compared to the pre-assessment.

Selection of Pedagogy

The development of the biotechnology curriculum for this study will, through a series of exercises organized into a broader scaled unit, take the students from the first level of inquiry, confirmation, and on to level two and three, structured guided, levels of inquiry described by Bell, Smetana, and Binns (2005). The curriculum will begin with students confirming basic principles and background knowledge, such DNA and the concept of genes and inheritance, through staging activities. It progress into activities where there is the opportunity for students to both devise their own procedure and investigate questions through a procedure provided by the teacher regarding gel electrophoresis. Classifying the curriculum by Hanegan and Bigler's (2009) definitions, the unit is of a structured and guided simple nature. The research question will be provided for the student and the protocol and data collection will be predetermined, though there will be some student exploration in each area. It has been noted that all forms of inquiry have their place in the science classroom and educational goals are diverse (Hanegan & Bigler, 2009; NRC, 2000).

The purpose in selecting this form of inquiry is to provide both students and teachers with a level of comfort while introducing aspects of a new educational method. Again, this research is more concerned with science knowledge and content and the mastering of scientific skills. Because one could not reasonably expect students to create their own procedure for a technique like polymerase chain reaction (PCR), structured and guided inquiry is more appropriate for both these students and the educational goals of this study (Blanchard et al., 2010). However, once the

procedure is explored, students will be able to implement it themselves to answer their own questions. A detailed description of the lesson plan and rationale for its development can be found in chapter three.

Delimitations

In this study, an inquiry-based biotechnology curriculum will be incorporated into an existing local Early College program targeted toward upperclassmen high school students who are in good standing and interested in pursuing a college degree in the medical and STEM fields. Students are solicited from both urban and rural public, private, and home-schooled environments located within a particular county within a Midwestern state. The generalizability of this study will be limited to other such populations.

Definition of Relevant Terms

Borrowing from the definitions used by Bigler and Hanegan (2011):

- Biotechnology is defined as the creation of products beneficial to humans through the use of organisms or part of organisms.
- Biotechnology topics include genes and heredity, genetic mutations, DNA fingerprinting, and forensics, among others.
- Biotechnology equipment and techniques includes micropipettes, centrifuge, vortex, agarose gel and gel electrophoresis, polymerase chain reaction (PCR), and thermal cyclers.
- Traditional learning is defined as a classroom where content standards are taught primarily through lectures and worksheets.
- Early College is a high school initiative whereby they partner with colleges and universities to offer students the opportunity to earn credit toward both their high school

diploma and an undergraduate degree at no or low cost to the student (American Institutes for Research, 2013).

- Inquiry-based instruction has roots in constructivism and emphasizes the use of student questioning, investigating, and problem solving, similar to the process of scientific inquiry, as the primary pedagogical strategy (Bybee, 2004).

Chapter II - Literature Review

Introduction

Inquiry has been a part of education since the days of Socrates. However, inquiry in American school systems has been present for less than a century (Bybee & DeBoer, 1993). Prior to the 20th century, education was viewed as a body of knowledge to be passed through direct instruction from teacher to student. The content of this literature review includes theoretical and empirical information that provides the background necessary for understanding the key principles of inquiry-based teaching and learning. It begins by presenting a historical context, starting when inquiry-based instruction began to gain popularity in the United States during the mid-1900s. It then addresses the conceptual framework through which this research is viewed, constructivism, and explores the characteristics of inquiry-based instruction, contrasting it to traditional teaching methods. Following this section, the literature review focuses on inquiry-based instruction in science education, exploring both the challenges and benefits of this method of instruction. Next, parallels are made to the challenges of implementing biotechnology education in secondary education and the relationship between biotechnology education and inquiry-based instructional methods is examined. This chapter then concludes with a summary which identifies the unique intersection between biotechnology education and inquiry-based instruction, and the need for further research in this area.

Historical Context

Inquiry-based instruction began to gain popularity in the United States during the discovery learning movement in the 1960s (Bruner, 1961). Similar to Karplus' Learning Cycle, Joseph Schwab (1960, 1966) suggested that science be presented as inquiry, and that students should work in an inquiry-based laboratory before being introduced to formal instruction on the

topic, allowing students to build explanations from evidence. Many curricula, such as that released by the Biological Sciences Curriculum Study around this time, included components of inquiry and were an attempt to turn the traditional "cookbook" approaches into more hands-on models that would include scientific investigation and reasoning (McComas, n.d.).

In 1983, a *Nation At Risk* was published by the National Commission on excellence in Education detailing the shortcomings of American education. This publication and related conference recommended all educational institutions adopt more rigorous standards and higher expectations. Many groups such as the American Chemical Society (ACS), the Technical Education Resources Center, the Lawrence Hall of Science, the National Science Resources Center (NSRC), and the Education Development Center responded to this report by developing new science curricula throughout the 1980s which incorporated the recommendations for content, standards, and expectations (National Research Council, 1996). Regarding the teaching of science specifically, the Commission encouraged the method of scientific inquiry and reasoning and cited that graduates should be equipped to both apply scientific knowledge to everyday life and understand the implications of scientific development (U.S. National Commission on Excellence in Education, 1983).

In the early 1990s, with the support of the National Science Teachers Association, National Academy of Sciences, National Research Council, the U.S. secretary of education, the assistant director for education and human resources at the National Science Foundation, the co-chairs of the National Education Goals Panel, and many other presidents of science education associations, the National Committee on Science Education Standards and Assessment (NCSESA) was established and was charged with overseeing the development of science education standards (NRC, 1996). By 1996, the committee released the *National Science*

Education Standards which emphasized the centrality of inquiry for learning science, so much so that *science as inquiry* is one of the eight categories into which the standards are divided. Within this category, the *Standards* further elaborate that "inquiry is a step beyond 'science as a process'" (NRC, 1996, p. 105). Rather, inquiry requires the combination of the process of science and scientific knowledge with the use of scientific reasoning and critical thinking. By engaging in all these processes in combination, students will uniquely develop their understanding of science. The *Standards* agree that inquiry facilitates a stronger understanding of scientific concepts and the nature of science, while helping students develop an appreciation for how scientific knowledge is constructed. Inquiry also nurtures the skills, abilities, and attitudes associated with science and the dispositions to use them and become independent inquirers about the natural world (NRC, 1996).

Running parallel to the release of the *National Science Education Standards*, and since their release of *Science for All Americans*, the AAAS initiative also developed *Benchmarks for Science Literacy* which was published in 1993. This document did not provide curriculum, but it did provide educators with learning goals from which they could design science curriculum. And although AAAS was careful to note that *Benchmarks* did not advocate for any particular teaching method, scientific inquiry was the second of three concepts addressed under the first benchmark; the nature of science. In this section, inquiry was described as a method that would provide students with an experience that would leave them with a more accurate image of the work done by scientists (AAAS, 1993).

This means that at this time the nation essentially had two national standards documents, the *Standards* and the *Benchmarks.* In 1998, the AAAS released another document, *Blueprints for Reform*, to help further the educational reform that would be needed for the successful

implementation of a new science curriculum. The National Research Council also released *Designing Mathematics or Science Curriculum Programs* and *Selecting Instructional Materials: A Guide for K-12 Science*, both in 1999. All of these documents were designed to help State- and district-level education leaders create curriculum programs that would help students learn science in a coherent and cumulative way throughout their schooling (Achieve, Inc., 2013). Yet, despite the attempts by these organizations, there was never really widespread incorporation of the standards across the nation (Horizon Research, Inc., 2013).

However, by the year 2000, the support for inquiry as the heart of science education remained strong and was reinforced in a publication by the National Research Council titled, *Inquiry and the National Science Education Standards: a Guide for Teaching and Learning*. In this document the public was reminded of the ability of inquiry instruction to engage students in the same thinking processes and activities that scientists practice in their efforts to expand human knowledge (NRC, 2000). However, the debate between inquiry and traditional instructional methods continued, especially after the No Child Left Behind (NCLB) Act was passed in 2001. Those who supported traditional teaching methods believed that other factors, such as teacher preparation time, teacher behavior, and school and teacher characteristics, had a greater impact on student achievement than the actual teaching methods employed (Schwerdt & Wuppermann, 2010). In October of 2004, the NSTA clarified their position in the debate when the group issued a statement which recommended “all K-16 teachers embrace scientific inquiry" and reinforced NSTA was "committed to helping educators make it the centerpiece of the science classroom" (National Science Teachers Association, 2004). Proponents of inquiry-based teaching methods found that such approaches generated increases both cognitively and affectively; students

experienced greater achievement outcomes and enjoyed the learning process more when compared to traditional methods (Herman & Knobloch, 2004).

In 2005, attention turned toward the laboratory, a component common for so many science courses, and how it could further contribute to the learning of science through inquiry when the National Research Council released *America's Lab Report: Investigations in High School Science*. This document was followed by publications from various organizations in 2007; *Taking Science to School*, *Rising Above the Gathering Storm*, and *Atlas for Science Literacy, Volume 2*. In *America's Lab Report*, the NRC reinforced the committee's view that, consistent with inquiry-based methods, effective science education included learning both the method and processes of research (science process), as well as the knowledge derived through this method (science content) (NRC, 2005).

The NRC suggests that laboratory experiences have the potential to offer an experience where students can engage in the learning of the scientific process - or scientific inquiry - along with scientific content. The committee had identified the following goals for laboratory experiences (NRC, 2005):

- Enhance student mastery of subject matter content.
- Develop student scientific reasoning skills.
- Develop student understanding of the complexity and ambiguity of empirical work.
- Develop practical skills.
- Develop student understanding of the nature of science.
- Cultivate student interest in science and in learning science.
- Develop student teamwork abilities.

A synthesis of the research by the NRC found improvements in both the development of scientific reasoning skills and an interest in learning science after students participated in traditional laboratory experiences. However, traditional laboratories were found to be no more or even less effective than other forms of instruction when assessed on the goal of mastery of subject matter content. Research on integrated laboratory experiences, laboratory exercises that emphasize the multidisciplinary nature of scientific research (Seybert, Evanseck, & Doctor, 2006), show greater effectiveness than traditional laboratories regarding the goals of mastery of subject matter content, development of scientific reasoning, and interest in science learning. Integrated laboratory experiences also appeared to be particularly effective in progressing diverse groups of students toward these goals. Based on the research, the NRC determined four principles of instructional design which allow integrated laboratory experiences to better achieve the identified goals (NRC, 2005): (1) meeting clear, predetermined learning outcomes, (2) thoughtfully integrating into the flow of instruction, (3) integrating the learning of both science content and the processes of science, and (4) incorporating regular student reflection and discussion.

Despite the many attempts to develop consistent standards, there was never really widespread incorporation of any standards across the nation. This is highlighted in the *2012 Survey of Science and Mathematics Education* (Horizon Research, Inc., 2013) that reported forty percent of science teachers across K-12 agreed "teachers should explain an idea to students before having them consider evidence for that idea" (p. 6). More than half indicated that laboratory activities should be used after new ideas had been learned, and nearly ninety percent were still using lecture and discussion as primary instructional activities.

Almost fifteen years after the release of the important work by the National Research Council and the American Association for Advancement in Science, the *Next Generation Science Standards* (NGSS Lead States, 2013) were introduced in an effort to update quality science education standards based on new understandings of how students learn science. This work was spurred by a 2007 Carnegie Foundation finding that "the nation's capacity to innovate for economic growth and the ability of American workers to thrive in the modern workforce depend on a broad foundation of math and science learning, as do our hopes for preserving a vibrant democracy and the promise of social mobility that lie at the heart of the American dream" (Carnegie Foundation, 2008, p. vii).

The interest in reexamining the standards was also based on evidence of lagging achievement of U.S. students. The United States ranked 17th in science on the 2009 PISA assessment of 34 OECD countries (Fleischman *et al*., 2010). Over a third of eighth graders scored below basic on the 2011 NAEP science assessment (National Center for Education Statistics, 2012), and in 2012, sixty-nine percent of graduates failed to meet the college readiness benchmark levels in science (ACT, 2012). The interest in reexamining the standards also stemmed from science as essential preparation for all careers in the modern workforce (National Association of State Directors of Career Technical Education Consortium, 2006) and the need for scientific and technological literacy for an educated society (White & Dillow, 2005). The NRC's 2011 publication, *Rising Above the Gathering Storm, Revisited* also warned that without the renewed effort to rebuild our competitive advantage, we would lose our positioning in the global landscape (Achieve Inc., 2013a).

An independent, nonprofit education reform organization, Achieve, Inc., was established in 1996, and in 2010 this group completed international benchmarking which would become the

foundation for the *Next Generation Science Standards*. Ten countries were selected based on either their strong performance on international assessments, their special interest to the United States, or both. Major findings of this study included all ten countries having integrated science standards rather than grade-level and subject-specific courses in grades six through ten, and crosscutting concepts common to all the sciences, such as the nature of science, which received substantial attention (Achieve Inc., 2010).

Another important finding was that inquiry skills were a cornerstone of the various curricula. The *International Science Benchmarking Report* (Achieve Inc., 2010) found that the development of inquiry skills led to increases in student interest in learning science and practicing scientific habits of mind. Canada stood out as a quality example with their system designed for progression from beginner to proficient in four main areas of both inquiry and design; initiating and planning, performing and recording, analyzing and interpreting, and communicating. However, the use of evidence-based inquiry was found to be a shortcoming in most other countries as five of the ten countries did not require students to focus on evidence during their science education. The College Board Standards, which were revised around this time, also called attention to the need to consistently incorporate the practice of establishing evidence and using that evidence to make predictions about natural phenomena, substantiate claims, and develop and test explanations. This shortcoming of other countries presented the United States with an opportunity to forge a fresh vision for science education (Achieve Inc., 2010).

Built upon the *International Science Benchmarking Report*, in 2012 the NRC published *A Framework for K-12 Science Education*, containing the three primary components that must be integrated for effective science instruction; disciplinary core ideas, science and engineering

practices, and crosscutting concepts (NRC, 2012). The next step was the development of the *Next Generation Science Standards,* a draft of which was completed in the summer of 2012. This was followed by several writing team reviews, State and critical stakeholder reviews, and public drafts before the final draft was released to the public in winter of 2013. After final edits were made to the documents, the *Next Generation Science Standards* were released for adoption in April of 2013 (NGSS Lead States, 2013).

Despite the fact that science instruction has not changed greatly over the last many decades, this historical context clearly illustrates that inquiry-based instruction has remained at the forefront of science education reform because of its potential to positively impact student outcomes. Thus, the design of any quality science curriculum must incorporate not only science content, but also the crosscutting concepts, and science processes necessary to help students develop both deep conceptual understanding and, as recommended earl on by Dewey, the skills and habits of mind of scientists.

Conceptual Framework

One key element to understanding educational theory is that of ontology, referring to issues concerning the nature of being and reality. Two contrasting branches of ontology are described by idealism which views claims of reality to be observer-dependent and not absolute, and realism which views the true nature of reality to be knowable independent of the observer (Hofweber, 2012). A second key element is epistemology, a branch of philosophy regarding the nature of knowledge, its origin, foundation, limits, validity, and its transmission (Costello & Botella, 2006).

For most of the 20th century, behaviorism was the dominant educational theory, which holds the view that learning is a process of stimulating learners to behave differently (Edgar,

2012). It is when the learner demonstrates a new behavior that it can be assumed the learning has occurred. The limitation of this theory is that there is little regard for what goes on inside the learner's mind, no time for evaluation or reflection within the process, and the teacher is the dominant person in the learning process. In contrast, constructivism, which stems from several lines of thought as early as "Ancient Greece and the questioning methods employed by Socrates" (Friesen, 2013, p. 6), views knowledge or reality as something to be created, or constructed, based on a learner's interactions with her or his surroundings (Oxford, 1997). This is in contrast to procedural approaches to knowledge, which view one reality as existing within the physical world, to be acquired by the learner (Manus, 1996).

Constructivism. Inquiry-based instruction has its roots in constructivism. Constructivist theory falls into two, nonexclusive, categories; 1) radical or cognitive constructivism and 2) social constructivism. Cognitive constructivism is founded on two claims; "(1) knowledge is not passively received, but rather actively built by the cognizing subject, and (2) the function of cognition is adaptive and serves the organization of the experiential world, not the discovery of ontological reality" (von Glasersfeld, 1989 p. 162). Therefore, based on these two claims, all experience is subjective. The mind organizes information into what is called "reality", based on the filter of individual biases, experiences and sensory perceptions. Social views of constructivism, on the other hand, emphasize that knowledge and reality are actively created through social relationships and interactions. Stemming from the work of Vygotsky and Bruner, social constructivism views knowledge as a social product and learning as a social process (Bruner, 1996; Vygotsky, 1978b).

As an educational approach, constructivism asserts that the learner creates meaning by selecting relevant pieces of information which are perceived through first-hand experiences, and

then builds upon personal knowledge to form a series of individual constructs, concepts, or models (Shuell, 1993). Beginning in the 1990s, constructivism was identified as the fundamental principle underlying science education (Matthews, 1998). Though two major areas exist, cognitive and social constructivism, which differ in emphasis, they share a common perspective regarding teaching and learning. Learning is an active process of constructing knowledge, and the teacher should support this construction of knowledge (Duffy & Cunningham, 1996). Knowledge cannot simply be transferred from one individual to another, but rather each individual must construct her or his understanding of the natural world through personal experiences and knowledge creation.

Giambattista Vico proposed the concept of constructivism early in a 1710 publication which described knowledge as something which was constructed by the knower. His slogan was "The human mind can only know what the human mind has made" (von Glasersfeld, 1989, p.3), and his works mostly looked at the relationship between truth and knowledge. However, Jean Piaget, a developmental psychologist, was viewed as the original constructivist as much of his work with child cognitive development and learning drew on the theoretical framework of constructivism. He supported a holistic approach to education, stressing that students learn through many avenues including reading and listening, but also exploring and experiencing their environments. Piaget is well-known for the development of his Four Stages of Cognitive Development. These stages include sensorimotor (birth to two years), preoperational (two to seven years), concrete (seven to eleven years), and formal operation (eleven years and into adulthood) (Piaget, 1964). Through these stages, children move from experiencing the world primarily through their movement and senses; to understanding the representation of meaning

through objects, words, and images; to conversing and thinking logically and away from egocentrism; and finally into the ability for abstract thought (Berger, 1988).

For Piaget (1964), learning cannot occur via the dissemination of information for immediate understanding and use; rather learners must construct their own knowledge through their experience. Experiences allow for the creation of mental models about the world. These models can then continually be changed through assimilation and accommodation, two complementary processes by which intellectual development occurs. Assimilation occurs when a learner responds to a new event that is consistent with an existing mental model, while accommodation occurs when a new event requires the modification of an existing model or the creation of a new model by the learner (Ormrod, 2012).

Lev Vygotsky was a psychologist and philosopher and was most commonly associated with social constructivism. Vygotsky often challenged Piaget's assertions regarding cognitive constructivism by stressing the importance of a student's cultural and social background on that individual's learning. Vygotsky theorized that learning occurs in two phases; first, between people at the social level (interpsychological), and then inside the learner at the individual level (intrapsychological). He supported the idea that all higher functions form as a result of the relationships between people. He is most notable for his theory of the Zone of Proximal Development (ZPD), which is the distance between what learners can achieve unassisted and what they can achieve with assistance (Chaiklin, 2003). Regarding science education, Vygotsky's view emphasizes the influence of the cultural and social contexts in learning. This model supports the importance of opportunities for students to engage with their peers and with 'experts'. Both interactions allow students the opportunity to scaffold, or build upon what they know to reach new levels of understanding they may not be able to reach unassisted.

Jerome Bruner is also regarded as one of the founding fathers of constructivist theory. Bruner's work emphasized the role of categorization in learning, as well as interpreting information and experiences by similarities and differences (Bruner, 1960). He also took a look at motivation and found that students were more likely to carry out their learning with greater autonomy when they are motivated intrinsically, such as by the desire to discover, rather than extrinsically, as is the case with grades or competition for class rank (Bruner, 1961). Bruner was influenced by Paiget's theories on cognitive development, but unlike Piaget, Bruner did not emphasize the different stages of development as separate modes of thought occurring at different points in development. Rather than seeing development in a linear model, Bruner believed development would follow a unique sequence for each individual. The particulars of this sequence would depend on a variety of factors such as previous learning, the nature of the material at hand, and other individual differences (Bruner, 1961). Bruner also shared similar beliefs with Vygotsky in that they both placed emphasis on the learner's environment, and the active role that a teacher should take in supporting the learner's acquisition of knowledge through experience (Bruner & Haste, 1987).

Urie Bronfenbrenner also supported the influence of the environment on learning with his Ecological Systems Theory (Bronfenbrenner, 1994). Bronfenbrenner held that individuals influence and shape social change over time while, simultaneously, individual knowledge, competencies, and development are influenced by the guidance, support, and structure provided by society at various levels. Bronfenbrenner's model divides the environment into "a set of nested structures" (Bronfenbrenner, 1994, p. 39); microsystems (direct environment), mesosystems (relationships between microsystems), exosystems (community), macrosystems (social conditions/culture), and chronosystems (transition over time). The microsystem is the

layer that includes family, friends, teachers, and the school environment, and the theory posits individuals are not mere recipients of experiences within this environment, but that individuals are contributing to the construction of that environment (Bronfenbrenner, 1994).

Aligned with these learning theories, Robert Karplus developed the Learning Cycle Approach of instructional design (Atkins & Karplus, 1962). In the Learning Cycle learners first explore a phenomena (exploration), they then are guided through an explanation of the phenomena where appropriate vocabulary is introduced (invention), and finally apply this knowledge to a new situation (application). This instructional approach emphasizes the investigation of phenomena through the design of experiments and the use of evidence in explanations (Abraham, 1982). The Learning Cycle is largely in contrast to more traditional modes of teaching where students are viewed as learning by receiving information from the instructor.

This study builds upon constructivism by harnessing a learner-centered approach through which knowledge is constructed. Activities are both independent and of a group nature so that learning occurs both cognitively, as described by von Glasersfeld, and as a social process, as supported by Vygotsky, Bruner, and Bronfenbrenner. The teacher serves as a guide while students construct knowledge as active participants in their own learning. Lessons are holistic, as Piaget suggested, and are offered through various avenues such as reading, writing, videos, and hands-on activities. Students are able to reflect on their own knowledge and then move toward testing their own ideas and drawing conclusions. They then combine their knowledge and communicate their findings to others through a collaborative learning environment.

Inquiry-based teaching and learning. Stemming from the theoretical root of constructivism, and a product of Bruner's discovery learning movement, inquiry-based

instruction developed in the 1960s in response to traditional methodologies. John Dewey was an early advocate for inquiry as the basis for scientific education (Hanegan and Bigler, 2009). In publications throughout the early 20th century addressing educational theory, Dewey drew from the early constructivists as he continuously cited education as a social and interactive process. Dewey characterized current cookbook lessons as "ready-made material with which student are to be made familiar (p. 183)", and identified the major deficiency of these educational practices as "not enough as a method of thinking, an attitude of mind, after the pattern of which mental habits are to be transformed (p. 183)" (Dewey, 1964). His contemporary, Paulo Friere, also rejected the banking model of education, which posits the learner as an empty vessel waiting to be filled with knowledge (Elias, 1994). Dewey and Friere both believed learning occurs best when students are allowed to experience the lessons and take part in their own learning by interacting with the curriculum. Inquiry-based instruction emphasizes the use of student questioning, investigating, and problem solving, similar to the process of scientific inquiry, as the primary pedagogical strategy (Bybee, 2004). According to the *National Science Education Standards* (NRC, 1996), five key features of inquiry-based instruction include; students engaged in scientifically oriented questions, students giving priority to evidence in responding to questions, students formulating explanations from evidence, students connecting explanations to scientific knowledge, and students communicating and justifying explanations. These key features are echoed in the *Science and Engineering Practices* that form a core element of the *Next Generation Science Standards* (NGSS Lead States, 2013):

1) "Asking questions (for science) and defining problems (for engineering)
2) Developing and using models

3) Planning and carrying out investigations
4) Analyzing and interpreting data
5) Using mathematical and computational thinking
6) Constructing explanations (for science) and designing solutions (for engineering)
7) Engage in argument from evidence
8) Obtaining, evaluating, and communicating information"

Inquiry-based instruction can occur either within a discrete activity or on a broader scale within the curriculum, and can range from quite structured and guided activities to independent research (Spronken-Smith *et al*., 2007; Windschitl, 2002). In 2005, Bell, Smetana, and Binns, while expanding on Herron's (1971) ideas, determined the following levels of inquiry (Ketpichainarong et al., 2010), where level one and two remain the predominant forms of inquiry in today's science classroom (Hofstein & Lunetta, 2004):

- Level One – Confirmation: Students are able to confirm basic principles through activities where the results are known.
- Level Two – Structured Inquiry: Students investigate questions through a procedure provided by the teacher.
- Level Three – Guided Inquiry: Students investigate the teacher's question through their own procedures.
- Level Four – Open Inquiry: Students investigate a topic by selecting their own questions and designing their own procedures.

Table 1. Four-Level Model of Inquiry.

Level of Inquiry	Question	Procedures/ Design	Results/ Analysis
Level 1 – Confirmation	X	X	X
Level 2 – Structured	X	X	
Level 3 – Guided	X		
Level 4 – Open			

The X marks the items or information provided by the teacher.

According to Hanegan and Bigler (2009), inquiry lessons can be categorized as either guided or open, and either simple or authentic. When the research question is provide for the student, she or he is performing simple inquiry rather than authentic inquiry, during which the student would generate the research question. Once the research question is determined, the choices regarding protocol and data collection can be predetermined and directed, as in guided inquiry, or determined by the student during open inquiry. Both open-ended and highly-structured methods have their place in the science classroom. Since both students and educational goals are diverse, the form of inquiry used should depend largely on these factors (NRC, 2000). Structured simple inquiry provides the basics of investigation to students, and may be beneficial when new techniques or procedures need to be introduced for more complicated investigations to occur later. Guided inquiry may be more appropriate once procedures are familiar to students and there is flexibility regarding the outcome of the investigation. Open inquiry most closely resembles the work of actual scientists, but may depend on the ability of the

teacher to facilitate student inquiry and the comfort of the student with the process (Zion & Mendelovici, 2012).

The following table offers a comparison between two pedagogies, contrasting characteristics between inquiry-based instruction and the traditional approach to student learning.

Table 2. Inquiry-based teaching versus traditional teaching.

	Inquiry-Based Teaching	Traditional Teaching
Overall	Student-centered	Teacher-centered
Guiding Learning Theory	Constructivism	Behaviorism
Curriculum Goal	Process oriented	Product oriented
Student Expectations	Active participation in process	Passive participation in process
	Increased responsibility	Decreased responsibility
	Problem solving role	Direction following role
Teacher Expectations	Guiding or facilitating role	Director or transmitter role
Resources	Expanded	Restricted
	Literature-based approach	Content-focused workbooks
Environment	Cooperative discussion	Silent, individual work
Learning Methods	Hands-on manipulations	Rote practice & memorization
	Connect to real-life meaning	Symbolic meaning

As indicated in Table 2, traditional approaches to student learning are teacher-centered and focused on the mastery of content. The teacher is viewed as the expert in the classroom and information is transmitted from the teacher to the student who passively engages in the process

of receiving that information. The approach is based on behaviorism, a learning theory that believes human behavior can be explained and altered in terms of conditioning (Edgar, 2012). This is why traditional classrooms often feature learning methods of rote practice and memorization. In traditional classrooms, students are expected to follow directions and have little responsibility for their own learning. The work is often completed silently and individually, and resources are restricted to those provided within the classroom. Reading materials are limited and students work from texts designed to drill them over the content on which they will be tested (Khalid & Azeem, 2012).

Inquiry-based approaches to student learning are student-centered and focused on the mastery of process. The teacher is viewed as another resource in the classroom and guides or facilitates learning for the student who is actively engaged in the process of knowledge construction. The approach is based on constructivism, a learning theory that states knowledge is something constructed actively by a learner in relation to her drive to understand her environment (Duffy & Cunningham, 1996). This is why the inquiry classroom features instructional methods focused on hands-on manipulations and questioning. In inquiry classrooms, students are expected to be problem solvers and have primary responsibility for their own learning. The work is often collaborative and group oriented, and resources are broad and technology helps to expand outside the classrooms. Reading materials are also expanded and multiple sources help to diversify the curriculum (Khalid & Azeem, 2012).

Inquiry-based instruction is preferred to traditional instruction because inquiry is more focused on the development of both information-processing and problem-solving skills by emphasizing how we come to know what we know rather than simple knowledge acquisition. Inquiry instruction also features more active student involvement through hands-on

investigations and student questioning. Increased student interest and engagement allows for more in-depth construction of knowledge surrounding the subject. Inquiry classrooms are also open systems where students are encouraged to use resources beyond their classroom and school to help them investigate problems and develop conclusions. Stock lesson plan are replaced with facilitated learning plans. These allow for slight deviations in the learning process while keeping important learning outcomes in focus. Inquiry classrooms also feature assessments that determine skill development in addition to content understanding, and which are focused on both in-school success as well as life-long learning preparation (WNET Education, 2004).

The biotechnology unit at the heart of this project is built on the principles of structured and guided inquiry-based instruction. Some scholars argue that anything but open inquiry is insufficient for developing critical and scientific thinking (Zion & Mendelovici, 2012). However, because this study is more concerned with science content and the nature of scientific knowledge, and perhaps primarily, the mastering of scientific skills, as well as developing foundational inquiry skills, structured and guided inquiry is more appropriate for both these students and the educational goals of this study (Blanchard et al., 2010).

Inquiry in Science Education

While most of the science education research literature supports the use of inquiry-based instruction, there are two studies that are commonly cited as evidence for using traditional instruction over inquiry-based instruction. Klahr and Nigam (2004) conducted a comparative study of 112 3rd and 4th grade students who were tasked with learning a new technique and then assessing this technique in others. Materials, goals, examples, explanation, and pace were all teacher controlled in the traditional classroom while there was no teacher intervention in the inquiry-based classroom beyond the initial suggestion of a learning goal. Students in the

traditional classroom were found to better master the new technique when compared to those students in the inquiry-based classroom.

Similarly, Kirschner, Sweller, and Clark (2006) in their review of the literature surrounding inquiry-based instruction also concluded "that there is no body of research supporting the technique" (p. 83). However, these researchers grouped together all forms of inquiry-based instruction, including discovery learning, problem-based learning, and experiential learning, and characterize them all as offering minimum guidance during instruction (Blanchard et al., 2010). They also argued that this approach ignores the structures that constitute human cognition which require teacher guidance in student learning (Kirschner, Sweller, & Clark, 2006).

What both of these studies fail to consider is that what they are describing most closely resembles Level 4 or open inquiry (Hanegan and Bigler, 2009; Ketpichainarong *et al*., 2010). Advanced levels of inquiry such as these require prior knowledge and skills in both the content area and inquiry, and are therefore only appropriate for certain groups of students (Zion & Mendelovici, 2012). Dean and Kuhn (2006) performed a follow-up study to Klahr and Nigam (2004), following students the same age over a 10 week period. Forty-five 4^{th} grade students were divided into three groups; 1) conducting only Level 4 or open inquiry, 2) direct instruction prior to investigation, and 3) direct instruction without engagement. In reviewing results of both an immediate posttest and another assessment given five weeks later, Dean and Kuhn found that direct instruction was neither necessary nor sufficient for meeting learning outcomes. Kirschner, Sweller, and Clark (2006) are right; hands-on activities alone are not enough for many students. Moreover, discovery learning or open inquiry is generally not appropriate for students with limited background knowledge. Rather a minimum level of guidance is required to engage

students in the process of learning and to reap the gains of inquiry instruction (Dalton *et al*., 1997).

Challenges of inquiry-based teaching and learning. As is highlighted by the research of Klahr and Nigam (2004) and Kirschner, Sweller, and Clark (2006), though science educators express great enthusiasm for inquiry-based instruction (Koballa, 2008), the lack of implementation is often explained by a lack of understanding on the part of the teachers (Alberts, 2000; Radford, 1998). Many teachers still incorrectly view inquiry as simply allowing students to do hands-on activities. Thus, for successful implementation, instructors need sufficient professional development to help them better understand inquiry instruction as well as sustained support (Blanchard et al., 2009; Crawford, 2000, 2007; Luft, 2007; Windschitl, 2004). Furthermore, many instructors view inquiry-based instruction as an approach that requires significant time and materials to develop and more time and effort on the part of the students (Moss, 1997) and a method that is difficult to manage in traditional classroom environments (Barab & Luehmann, 2003; Henry, 1996; Stake & Easley, 1978).

Regarding science education in general, reform efforts in support of constructivist, inquiry-based approaches have a long history and have resulted in the development of national goals in the form of the *National Science Education Standards (NSES)* (NRC, 1996) and more recently the *Next Generation Science Standards* (NGSS Lead States, 2013). Despite the extended history, research regarding implementation suggests erratic attempts that result in incongruence between theory and practice (Bybee, 1997; Cochran-Smith & Lytle, 1990; Hurd, 1991). One reason for this is no doubt the challenges associated with the successful implementation of inquiry-based instruction, especially those lessons of an authentic and open-ended nature (Anderson & Helms, 2001; Blumenfeld et al., 1991; Crawford, 2007). Critics cite issues such as

a lack of aligned curriculum materials, the need for extensive professional development, the paucity of assessments capable of measuring new performance expectations, a lesser emphasis on science instruction, and the unique needs of schools which serve low-income students as hurdles to implementation (Penuel, Harris, & Haydel DeBarger, 2015).

More specifically, in their research on climatology education and inquiry through visualization, Edelson, Gordin, and Pea (1999) identified five main challenges to the successful engagement of students in inquiry-based activities. 1) Because inquiry learning is often more challenging when compared to traditional learning activities, a higher level of motivation may be demanded of the learner. 2) The success of inquiry learning also depends on student access to required investigation techniques – an ability to perform the technique, as well as to collect and interpret data. 3) Beyond the technique, learners must also have the necessary background knowledge to successfully carry out an entire investigation. This requires a learner to have knowledge not only in the subject matter, but also general scientific knowledge in the areas of formulating research questions and developing a research plan ahead of the collection, analysis, and interpretation of data. 4) When compared to traditional methods, inquiry instruction also requires extended activities. This again poses challenges for maintaining motivation but it also requires learners to manage those activities, through planning and coordination, in a way that traditional activities have not held learners responsible. 5) Inquiry-based instruction may not fit within the technological, financial, or schedule limitations of the learning environment. As solutions to these challenges the authors propose the following:

1) Select a meaningful problem which will have implications that matter to students as a way of establishing motivation.

2) Design bridging activities which will "bridge the gap between the practices of students and scientists" (p. 401), allowing them to gain the investigation techniques they will need for further learning.
3) Set the stage for open-ended inquiry by building in structured staging activities or investigations which will provide the background knowledge needed for later stages of learning.
4) Take advantage of various forms of technology to provide supportive user interfaces and embedded information sources to students. These systems provide immediate access to information needed to complete an investigation and the scaffolding necessary for learning from experts.
5) Provide various record-keeping tools. These will help learners progress through extended learning activities by supporting the management and organization of inquiry as well as maintaining motivation.

Similar to Edelson, Gordin, and Pea (1999), in their studies regarding the implementation of inquiry-based instruction in geography, Spronken-Smith, Bullard, Ray, Roberts, & Keiffer (2008) cite the perception of an increased time commitment as a primary hurdle for students, as well as problems with group work and a lack of security in both format and content. Spronken-Smith, *et al*. (2008) suggest educators may mediate these hurdles by addressing them at the forefront of instruction, by providing students with the purpose and goals of inquiry-based instruction and by allowing students to determine how their groups will function effectively. By explaining the benefits of inquiry-based instruction to students, educators may be able to ameliorate the reservations students have toward this type of learning activity.

Spronken-Smith, *et al.* (2008) also explain educators may face similar challenges with insecurity. Even after experiencing inquiry-based teacher education programs, most teachers return to traditional instructional practices (Schneider et al., 2005). Moreover, of the small percentage of instructors who do use inquiry-based curricular materials, many end up delivering a watered down version with limited hands-on experiences (Fishman & Krajcik, 2003; Hubermann & Middlebrooks, 2000; Welch *et al*., 1981). On average, fifteen percent of class time in elementary schools and only two percent in high schools is spent on inquiry-based instruction (Weiss et al., 2003), and at both the state and district level, the majority of learning outcome statements are still content-oriented (Eltinge & Roberts, 1993; Welch *et al*., 1981).

Instructor insecurity may result from frustration or difficulty in knowing when to intervene with a student, which may become exaggerated when there are disparate levels of intervention in a team teaching scenario. Anxiety may also occur over the format of instruction since inquiry-based learning activities may appear to have a lack of structure or be less predictable in nature when compared to traditional teaching methods (Simon & Schifter, 1991). This becomes exacerbated when teachers are uncomfortable with the content and becomes more difficult because most teachers have been taught themselves via traditional pedagogy (Burns, 2007; Windschitl, 2004). These are both key concerns for inquiry-based teaching in the field of biotechnology (Bigler & Hanegan, 2011; Gengarelly & Abrams, 2009).

To address these challenges for educators, Spronken-Smith, *et al.* (2008) provide guidance in the selection of the type of inquiry-based teaching technique to be used, in managing a teaching team, as well as advice in facilitating learning in this technique. Other researchers also focus on the solution of curriculum design (Marx, 2003; Marx, *et al*., 2004; Singer, Marx, Krajcik, & Clay-Chambers, 2000) and learning technologies (Krajcik *et al*., 2000; Marx *et al*.,

2004; Soloway, Guzdial, & Hay, 1994) similar to Edelson, Gordin, and Pea's (1999) focus, as well as opportunities for professional development since teachers cannot be expected to move directly to inquiry approaches from more traditional instruction (Fishman, *et al*., 2003; Marx *et al*., 2004).

Benefits of inquiry-based teaching and learning. Despite the challenges discussed above, inquiry-based instruction has been at the forefront of science education reform documents for the past two decades because it has numerous benefits for students including increases in both cognitive and affective outcomes (Herman & Knobloch, 2004). The National Science Teachers Association (NSTA) recognizes inquiry-based instruction as a form of learning that is more aligned with both how scientists conduct science and a student's natural curiosity and interest in interacting with her environment. The NSTA also supports inquiry-based instruction as a way of learning both science content and process, as it includes asking questions and using evidence to find answers through investigation and the collection of data, the formation of an explanation from that data, and the communication and defense of those findings (NSTA, 2004).

Although motivation is often cited as one of its challenges, inquiry-based instruction has the ability to motivate students for their own learning, regardless of whether the proper result occurs. Inquiry provides an opportunity for students to be actively engaged in the process and be driven by the desire to find an answer (Wilke & Straits, 2005; Witt & Ulmer, 2010). Motivation increases when tasks are shown to be relevant and challenging but at the proper level of difficulty for the learner, so as to neither bore nor frustrate the student (Bransford, Brown, & Cocking, 2000). Through successful engagement, inquiry promotes student ownership of current learning and increases interest in future inquiry (Haury, 1993; Wilke & Straits, 2005; Witt & Ulmer, 2010).

In contrast to traditional methods, inquiry provides a means for students to construct their own understanding and question knowledge (Perry, 1970; Wilke & Straits, 2005). Rather than teaching students how to memorize and regurgitate facts, inquiry develops the skills needed for students to become life-long learners (Witt & Ulmer, 2010), promoting the learning that is demanded by 21st century skills, in a world where knowledge and information is continuously emphasized and growing at an exponential rate (Pacific Policy Research Center, 2010). Consistent with the *Framework for 21st Century Learning* (Partnership for 21st Century Learning, 2007), inquiry nurtures intellectual development in the area of increased critical thinking and problem solving skills, facilitates creativity and innovation, encourages collaboration with others, strengthens communication skills, and prepares students to become informed members of society, especially in the areas of technology, health, and environmental literacy (Pacific Policy Research Center, 2010).

Inquiry-based instruction also results in a student's deeper understanding of scientific concepts rather than just the acquisition of skills (Anderson, 2002; Colburn, 2004; Ertepinar & Geban, 1996; Krajcik et al., 2000; Shymansky, Kyle, & Alport, 1983; Witt & Ulmer, 2010; Von Seeker & Lissitz, 2002). Even in elementary classrooms, on average, students instructed using inquiry-based methods outperformed students from traditional classrooms at least moderately in the areas of science process and science content, and the benefits were most pronounced for disadvantaged students, as concluded by Bredderman (1982) in a meta-analysis. In a review of studies across elementary, middle, and high school classrooms, Shymansky, Kyle, and Alport (1983) also found increased performance by students who studied science via inquiry methods. Von Secker and Lissitz (1999) found increased achievement to be highest in classrooms with

combined focus on problem solving, extended study, science writing, student interest, and lab work.

The vast majority of research studies focusing on the impacts of inquiry-based instruction have identified improved student learning (Berns & Lawton, 2004; Duschl, Shouse, & Schwingruber, 2007; Jorgenson, 2005; Lumpe, Czerniak, & Haney, 1999; Price & Felder, 2007). In 1983, Ted Bredderman published a study which evaluated the effectiveness of three activity-based elementary science programs; the Elementary Science Study (ESS), Science - A Process Approach (SAPA), and the Science Curriculum Improvement Study (SCIS), which were selected due to their popularity with the National Science Foundation as part of an inquiry Synthesis Project. Bredderman's meta-analysis found gains in all outcome areas which included science process tests, science content, affective outcomes, creativity, intelligence, language, and mathematics. The study also found that disadvantaged students derived the greatest benefits (Bredderman, 1983). As a continuation of this Inquiry Synthesis Project, Minner, Levy, and Century (2010) sought to synthesize findings from studies conducted from 1984 to 2002 to answer the research question, "what is the impact of inquiry science instruction on K-12 student outcomes" (p. 474)? Of the 138 studies in the synthesis, fifty-one percent showed positive impacts on content learning and retention resulting from some level of inquiry instruction. The study also found that science conceptual learning increased when students engaged in active thinking and participation in the investigation process.

Other earlier reviews show similar results. For example, in reviewing student outcomes in K-12 classrooms, through a systematic analysis of 105 experimental research studies on 27 inquiry-based curricula, Shymansky, Kyle, and Alport (1983) found students from inquiry-based science classrooms performed better on achievement tests than those who studied science in

traditional classrooms. Higher achievement scores for inquiry-based science instruction were also found by Von Secker and Lissitz (1999) in their analysis of data collected from the 1990 National Education Longitudinal Study (NELS) from 7,642 10th grade students. They found that the strongest results occurred in those classrooms which were able to simultaneously emphasize student interest, problem solving, lab work, depth of study, and scientific writing. Schnieder, Krajcik, Marx, and Soloway (2002) tracked 10th and 11th graders who were taught using inquiry-based instruction. Looking at their performance on the 1996 12th grade NAEP science test, the researchers found these students significantly outscored their peers on 44 percent of the test items, and the greatest difference was found on the extended constructed response questions where these students scored higher than the national average on 75 percent on the questions.

Other research on inquiry-based instruction and high school student performance includes that of Wilson, Taylor, Kowalski, and Carlson (2010) in which students were randomly assigned to either an inquiry or traditional classroom and both were taught by the same instructor based on the same learning outcomes. Student performance was measured using pre and posttests and the inquiry-based instructional group was found to outperform the students in the traditional class in all areas; knowledge, reasoning, and construction of scientific explanations. In interviews conducted four weeks later, in which student explanations were scored based on the quality of their claim, evidence, and reasoning, the inquiry students again scored significantly higher in all three dimensions compared to the traditional classroom students. Also, an achievement gap was found by race in the traditional classroom, but was nonexistent in the inquiry-based class (Wilson *et al*., 2010).

The evidence thus far supporting the benefits of inquiry-based instruction has direct implications for the work of this study; however, as we are developing a curriculum for a pre-

college course it is also important to note that inquiry-based instruction has also been shown to be beneficial at the undergraduate level. Casotti, Reiser-Danner, and Knabb (2008) compared students' performance on the presentation part of a physiology curriculum final assessment. Physiological content, scientific approach, and critical thinking were the outcomes being measured. The presentations of students after a curriculum shift was made toward inquiry-based instruction were found to score significantly higher in the areas of scientific approach ($p < 0.001$) and critical thinking ($p < 0.01$), and student understanding of physiological concepts was near significant ($p = 0.06$). Content gains resulting from inquiry-based instruction were also reported by Lewis and Lewis (2008) in their study examining outcomes of students in undergraduate general chemistry courses. Data was collected from sixteen sections using peer-led guided inquiry and thirteen traditional sections spanning three years, totaling 2,838 students. This total was reduced to 1,747 students whose SAT scores and American Chemical Society (ACS) exam scores could be collected. The researchers found increased performance, 1.19 points higher on average ($p < 0.001$), on the ACS exam for those students taught using inquiry-based instruction, regardless of student or class average SAT scores.

Barriers to Incorporating Biotechnology Education in High Schools

Biotechnology education is a current and relevant topic that should be explicitly addressed. It has grown rapidly as a field and now, in the 21st century, plays a critical role in several industries including agriculture and medicine (Klop et al, 2010). This scientific revolution demands a need for a scientifically literate population which is capable of making informed decisions regarding issues that reach their daily lives, careers, and society as a whole (National Academy of Sciences, 1996). Although the *National Science Education Standards* (NRC, 1996) do not specifically mention biotechnology, they do emphasize, in their *Life Science*

standards for grades 9-12, that "molecular biology will continue into the twenty-first century as a major frontier of science" (p. 181) and students should be able to make "informed positions on some of the practical and ethical implications of humankind's capacity to manipulate living organisms" (p. 181). *A Framework for K-12 Science Education* (NRC, 2011) does specifically mention biotechnology topics in its section on engineering, technology, and applications of science.

Despite its growing significance in our world and the fact that it is explicitly mentioned in *A Framework for K-12 Science Education*, inclusion of biotechnology topics in high school science classes is still minimal at best (Borgerding, Sadler, & Koroly, 2013; Hanegan & Bigler, 2009; Steele & Aubusson, 2004). Research indicates that this lack of inclusion is not due to lack of interest. Surveys of teachers in the US, Australia, and South Korea all show that teachers believe biotechnology education to be important and want to spend more time on the topic in class (Brown *et al*., 1998; Kwon and Chang, 2009; Steele and Aubusson, 2004). Moreover, a study by Michael *et al*. (1997) in which focus group interviews of sixteen Irish biology teachers were conducted, found teachers believe biotechnology to be engaging and motivational for students. So, the question becomes, why hasn't biotechnology education been widely implemented within the science curriculum? Borgerding, Sadler, and Koroly (2013) conducted interviews with twenty life science teachers in attendance at a biotechnology institute using protocol specifically designed to target the Stages of Concern framework modified by Hord *et al*. (1987). Their findings suggest that there are four major barriers for teachers in implementing biotechnology instruction:

1. Teachers desire more information regarding biotechnology.

2. Teachers' personal perceptions of biotechnology may impact their instructional decisions.
3. Teachers' concerns regarding student impact may prevent the implementation of biotechnology instruction.
4. Teachers are concerned regarding where biotechnology may fit in the curriculum or how much emphasis should be placed on the subject given current accountability systems.

Teachers desire more information both related to subject matter knowledge and instructional practices and curricula (Borgerding, Sadler, & Koroly, 2013). In their focus groups, Michael et al. (1997) found that even biology teachers consider themselves lay-people with respect to biotechnology, and when asked about what biotechnology entails and what should be taught, the results were quite broad and varied (Borgerding, Sadler, & Koroly, 2013). Leslie and Schibeci (2003), in their survey of self-selected participants who attended professional development presentation at Murdoch University, found teachers' conceptions of biotechnology fell into four broad categories: past and current technologies; gene technology alone; technology as applied to biology; and anything related to medical science. When considering what should be taught, teachers cited the following topics, many of which are typically found in general biology courses: DNA structure and function; genes and heredity; adaptation and speciation; Mendelian genetics; chromosomal theory; and cell biology (Borgerding, Sadler, & Koroly, 2013). Biotechnology-specific topics such as bioethics, applications within medicine, the environment, and agriculture, career opportunities, advantages and limitations of biotechnology, and the impact on humans were referenced only minimally (Brown et al, 1998; Zeller, 1994). When asked about how they deliver biotechnology instruction in their classrooms, teachers most often

cited lecture or discussion methods (32.6%), followed by laboratories (21.4%), and audiovisual methods (17.5%) (Zeller, 1994).

Teachers' personal perceptions of biotechnology may impact their instructional decisions (Borgerding, Sadler, & Koroly, 2013). In their study of 114 Korean middle school technology teachers through an online self-piloted survey, Kwon and Chang (2009) found a negative correlation between years of teaching experience and teaching biotechnology. However, in their survey of 135 science teachers (including 11 interviews and 2 case studies within that population) in New South Wales, Australia, Steele and Aubusson (2004) found no relationship. These authors also found no relationship between degree type and biotechnology instruction (Steele and Aubusson, 2004). Instead, studies have shown teachers hold a generally positive view of biotechnology (Kwon & Change, 2009), or at the very least, welcome modern advances with some reservations (Michael et al, 1997). Rather, teachers' attitudes toward biotechnology were found to be most positively associated with further training, professional development, and biotechnology instruction (Steele & Aubusson, 2004; Zeller, 1994). Therefore, teachers' reluctance to incorporating biotechnology into their curriculum exists in relation to how much time may be needed to adjust their current teaching practices (Borgerding, Sadler, & Koroly, 2013). Biotechnology education literature further supports this hindrance by consistently documenting concerns that fall into three related categories: lack of funding for materials and equipment (Zeller, 1994); scarcity of hands-on instructional materials (Steele & Aubusson, 2004); and lack of adequate preparation and instructional time (Michael et al., 1997; Steele & Aubusson, 2004; Zeller, 1994).

Teachers may also have concerns regarding students' response to biotechnology topics which may discourage them from including such topics in their curriculum (Borgerding, Sadler,

& Koroly, 2013). In various studies, teachers have reported concerns regarding student engagement with biotechnology (Reicks et al., 1996). Teachers worry about addressing ethical issues related to biotechnology (Bryce & Gray, 2004; Michael et al., 1997), and teachers face concerns regarding student frustration with conceptually-demanding material (Steele & Aubusson, 2004). Although researchers from several countries have determined students generally have a poor understanding of biotechnology (Chen & Raffan, 1999; Dawson, 2007; Dawson & Schibeci, 2003; Dawson & Soames, 2006; Gunter et al., 1998; Lock & Miles, 1993; Olsher, 1999; Ozel et al., 2009; Prokop et al., 2007; Turkmen & Darcin, 2007; Vennville & Treagust, 2002), biotechnology instruction is actually well received, and with high interest, by students (Bryce & Gray, 2004; Kidman, 2010; Ozel et al., 2009). Other studies have shown that, as a result of biotechnology instruction, students show increased interest and motivation for learning biotechnology (Dori et al, 2003; Hanegan & Bigler, 2009; Reicks et al., 1996), an increase in positive attitudes toward the biotechnology field as a whole (Klop et al., 2010), increased knowledge of biotechnology and its applications (Bigler & Hanegan, 2011; Dawson & Soames, 2006; Hanegan & Bigler, 2009, Reicks et al., 1996), improvements in higher order thinking (Dori et al., 2003; Olsher & Dreyfus, 1999), and an increased recognition of the importance of evidence (Bryce & Gray, 2004).

Concerns regarding where biotechnology may fit in the curriculum or how much emphasis should be placed on the subject given current accountability systems may pose another challenge to implementing biotechnology instruction (Borgerding, Sadler, & Koroly, 2013). In a study of Kentucky teachers in the area of science, technology, and agriculture, sixty-nine percent supported the teaching of biotechnology, while only thirty percent of the schools sampled offered it (Brown et al., 1998). However, if biotechnology is viewed as encompassing mostly biological

content, physical science teachers may not include it in their programming (Leslie & Schibeci, 2006). Quite often, biotechnology is omitted in favor of instructional time for other science-related content due to the need for preparation for external examinations (Steele & Aubusson, 2004).

In their study of Australian science teachers, Leslie and Schibeci (2006) found barriers that mirror those described by Borgerding, Sadler, and Koroly, (2013). The top five factors selected, by both biological and physical science teachers, as barriers to implementing biotechnology instruction include: "1) I have little or no personal knowledge of the content, 2) there is little or no information available at my school, 3) I don't know where to find suitable resources, 4) it requires expensive equipment to conduct labs, and 5) computer access in my school is difficult for whole classes" (Leslie & Schibeci, 2006, p. 101). However, their research also described factors which acted as encouragement to implementing biotechnology instruction. The top five agreed upon by both biological and physical science teachers included: "1) a package of materials available for immediate use in class, 2) simple concise resources for me to learn about biotechnology, 3) sample assessment items with marking rubrics available, 4) local examples that would be of interest to students, and 5) a range of activities that can be used individually and incorporated into existing units" (Leslie & Schibeci, 2006, p. 102). Their findings were consistent with the literature in that the barriers and encouragement factors could be explained in general terms of increasing teacher understanding and confidence regarding biotechnology education.

Inquiry-Based Teaching and Learning in Biotechnology

Research on the use of inquiry-based biotechnology instruction is very limited. However, what little is out there does support that it has the capacity to benefit biotechnology education in

much the same way that is has other science disciplines. In a study of 321 students in six high school biology classes using a microscopy and biotechnology curriculum redesigned collaboratively between high school teachers and university faculty to incorporate active-learning-based laboratory units, Taraban, Box, Myers, Pollard, and Bowen (2007) found that students using the inquiry-based curriculum performed significantly ($p < 0.001$) better in both the microscopy and biotechnology labs. In another study, Bigler and Hanegan (2011) found student content knowledge increased after a hands-on biotechnology intervention, Project Crawfish, was implemented in secondary biology classrooms. When comparing groups on the areas of DNA extraction/gel electrophoresis, PCR, DNA sequencing, bioinformatics, and phylogenetics, the ninety-three students in traditional classrooms only showed significant increases for PCR and DNA sequencing ($p = 0.0459$ and $p = 0.0043$, respectively), while the 125 students in the inquiry classrooms showed significant increases in all areas ($p = 0.0027$, $p < 0.0001$, $p = 0.0007$, $p = 0.0004$, and $p = 0.0128$, respectively). Bethel and Lieberman (2014) designed a multidisciplinary guided-inquiry biotechnology unit focused on the three-dimensional structure of proteins, their function, and connection to disease. At the time their article was published, the unit had been taught to eighty-two students, and cumulative comparisons of pre and posttests showed marked improvements in student achievement (36 +/- 15% on pre-tests compared to 80 +/- 11% on post-tests) in the areas of protein structure, the molecular basis of disease, and the scientific process.

As with inquiry-based instruction in science in general, inquiry-based instruction in biotechnology has also proven successful at the post-secondary level. In a university molecular biology course, through the incorporation of project-based learning, a form of inquiry-based instruction, students showed improvements in laboratory technical skills in the areas of cloning, transfection, expression, and protein purification (Movahedzadeh, Patwell, Rieker, & Gonzalez,

2012). In another molecular biology curriculum redesign, researchers found students made gains in technical skills areas such as bioinformatics and bibliographic searches, as well as cDNA templates and cloning vectors, polymerase chain reaction (PCR) amplification, and restriction and ligation reactions (Lesmes Celorrio, Fernandez Gomez-Chacon, & Gonzalez-Soltero, 2013). Conceptual understanding and technical skills were also improved in a cellulose-cellulase lab redesigned by Ketpichainarong, Panijpan, and Ruenwongsa (2010). Understanding of three main enzyme topics were significantly higher ($p < 0.001$) compared to pretest scores, and increases were highest in the application aspects and methods for measuring enzyme activity. Using inflammation in macrophages as a model system, Gunn, Seitz McCauslin, Staiger, and Pirone (2013) developed a structured inquiry-based biotechnology laboratory curriculum which resulted in ninety-five percent of their students successfully meeting learning outcomes in the areas of transfection and luciferase reporter assay, immunoblot, fluorescence microscopy, enzyme-linked immunosorbent assay, and quantitative polymerase chain reaction.

Incorporating inquiry-based instruction into biotechnology curriculum has also successfully led to gains in student attitude and motivation at both the secondary and post-secondary levels. For example, Klop et al. (2010) redesigned a science module on the topic of cancer and modern biotechnology based on social constructivist learning theory and conducted a quasi-experimental study regarding secondary school students' attitudes towards modern biotechnology. Questionnaires from 365 students were analyzed via chi-square and significant differences ($p < 0.05$) were obtained between control and constructivist classrooms, resulting in a more positive attitude toward modern biotechnology from the experimental group. The study by Movahedzadeh, Patwell, Rieker, and Gonzalez (2012) also supported increased interest in STEM-related fields, as well as improvements in student self-confidence. Student questionnaires

from the study by Taraban, Box, Myers, Pollard, and Bowen (2007) showed a preference for active-learning and that students perceived greater learning gains in biotechnology after completing the labs compared to traditional instructional methods. Though students, in the study by Lesmes Celorrio, Fernandez Gomez-Chacon, and Gonzalez-Soltero (2013), found time management of the projects to be the biggest challenge, they did find the ability to present their findings to be the most positive aspect of the process. Student assessments from another study also revealed, when inquiry-based instruction is incorporated with biotechnology education, students showed improved perceptions regarding personal relevance, scientific uncertainty, critical voice, and attitude, but not in the area of shared control. Student interviews in this same study revealed they felt more active in their learning, that topics were more relevant, and they had more opportunities to investigate their own problems, communicate their ideas and data with peers, and to draw their own conclusions through the use of their own evidence compared to traditional instruction (Ketpichainarong, Panijpan, and Ruenwongsa, 2010).

Summary

As has been reviewed here, inquiry-based teaching and learning dates back to the days of Socrates, but regained attention in the early 20th century when John Dewey wrote of his criticisms of traditional instruction (Bybee & DeBoer, 1993). In contrast to prior teacher-centered pedagogies, inquiry places the student at the center of her learning, recognizing that knowledge is constructed by the learner through interactions with her environment, rather than something to be passed from teacher to student. Inquiry is further supported by other learning theorists including Jean Piaget and Jerome Bruner (Bruner, 1960; Piaget, 1964).

Inquiry gained popularity during the discovery learning movement of the 1960s and began to be recognized in national curricula such as the Biological Sciences Curriculum Study

around this time (Bruner, 1961). In response to a *Nation At Risk* (U.S. National Commission on Excellence in Education, 1983), many educational groups continued to develop science curricula which included components of inquiry throughout the 1980s. And, by the early 1990s, many groups were calling for more rigorous standards leading to the development of the AAAS *Benchmarks for Science Literacy* in 1993, which included the concept of inquiry in its first benchmark, and the *National Science Education Standards* in 1996, which elaborated on inquiry as being a "step beyond science as a process" (NRC, 1996, p. 105). Though there was never widespread adoption of either of these standards, inquiry remained at the heart of science education, and is still a prominent concept in the most recent *Next Generation Science Standards* (Achieve, Inc., 2010).

Inquiry-based learning can occur either within a discrete activity or on a broader scale within the curriculum, and can range from level one – confirmation, to structured (level two) and guided (level three) activities to level four – independent research (Spronken-Smith *et al*., 2007; Windschitl, 2002). Most of the science education research literature supports the use of inquiry-based instruction (Berns & Lawton, 2004; Duschl, Shouse, & Schwingruber, 2007; Jorgenson, 2005; Lumpe, Czerniak, & Haney, 1999; Price & Felder, 2007), and those few papers that do not are often criticizing the problems that are encountered through the inappropriate selection of inquiry level for the learning objectives (Hanegan and Bigler, 2009; Ketpichainarong et al., 2010; Kirschner, Sweller, & Clark, 2006; Klahr & Nigam, 2004).

Despite the support, inquiry-based instruction is yet to be the norm is the majority of classrooms (Schneider et al., 2005). Though science educators express great enthusiasm for inquiry-based instruction (Koballa, 2008), the lack of implementation is often explained by a lack of understanding on the part of the teachers (Alberts, 2000; Radford, 1998). Many view it as

an approach that requires significant time and materials to develop and more time and effort on the part of the students (Moss, 1997) and a method that is difficult to manage in traditional classroom environments (Barab & Luehmann, 2003; Henry, 1996; Stake & Easley, 1978). Successful implementation is also inhibited by teachers' lack of confidence in their content knowledge, as well as their pedagogical and theoretical knowledge (Crawford, 2007).

The implementation of biotechnology education suffers from barriers similar to those encountered by the implementation of inquiry-based instruction in other science disciplines. Although biotechnology is a current and relevant field and biotechnology education is supported by the *Life Science* standards for graded 9-12 and the *Framework for K-12 Science Education* (NRC, 1996; NRC, 2011), the inclusion of biotechnology topics in high school science classes is still minimal at best (Borgerding, Sadler, & Koroly, 2013; Hanegan & Bigler, 2009; Steele & Aubusson, 2004). The lack of implementation can be explained by a number of factors: teachers' need for more information related to subject matter knowledge and instruction practices; teachers' perceptions about the subject may impact their instructional decisions; teachers' concerns regarding student impact; and teachers' inability to determine where biotechnology fits within the curriculum (Borgerding, Sadler, and Koroly, 2013).

Though research on the use of inquiry-based instruction in the area of biotechnology is limited, it does indicate that the benefits are similar to what they have been for other science disciplines. This review of the literature has shown there have been content knowledge and science process, as well as student satisfaction and subject area interest, benefits at both the secondary and post-secondary levels (Bethel and Lieberman, 2014; Bigler and Hanegan, 2011; Ketpichainarong, Panijpan, and Ruenwongsa, 2010; Klop et al., 2010; Lesmes Celorrio,

Fernandez Gomez-Chacon, and Gonzalez-Soltero, 2013; Movahedzadeh, Patwell, Rieker, and Gonzalez, 2012; Taraban, Box, Myers, Pollard, and Bowen, 2007).

Given that inquiry focuses on a student's ability to be at the center of their own learning, and the fact that biotechnology is a topic that requires hands-on learning for students to fully understand cell and molecular techniques, there is a unique intersection that allows these two aspects of education to work hand in hand. And, given the lack of widespread implementation of either of these, this intersection would benefit from further investigation. Lesmes Celorrio, Fernandez Gomez-Chacon, and Gonzalez-Soltero (2013) recommended steps in adapting introductory science courses to inquiry-based learning. First, identify a biological or medical problem which is both related to student interest and constitutes a key experimental objective for the course. Second, develop a research project that includes key skill development for the laboratory. Third, encourage students to be flexible and work on the protocol in order to improve results of the experiment. In a broad sense, these are the steps that have been taken in the following pages to implement an inquiry-based biotechnology unit for early college students.

Chapter III - Research Methods

Introduction

Given the many identified barriers to teachers' implementation of inquiry-based instruction, it was important to carefully consider these in the development of this curriculum. To ameliorate the challenges to implementing inquiry-based learning, the suggestions made by Spronken-Smith et al. (2008) were considered in the development of this study. First, careful attention was given to the type of inquiry-based teaching technique selected at each point in the biotechnology unit. Because inquiry, as well as the biotechnology content, is new to these students and this curriculum, the unit begins at the first level of inquiry and progresses on to guided inquiry. Also, the authors suggest prolonged exposure to inquiry-based instruction is more beneficial (Spronken-Smith et al., 2008), so these methods are integrated throughout the entire three-week unit rather than in just one assignment. Second, since the course was to be taught by both the researcher and the resident instructor as a teaching team, both were involved in all stages of planning the inquiry-based learning unit (Spronken-Smith et al., 2008). The curriculum was developed and first run as a pilot so the logistics of administering it could be assessed, and adjustments were made to the timeline and protocol for the laboratory activities. The researcher and resident instructor worked closely throughout this entire process. Finally, adjustments were made to the curriculum and pedagogy to allow for the incorporation of activities required in order to properly facilitate an inquiry-based learning unit (Spronken-Smith et al., 2008). Information regarding the unit, expectations for this type of learning environment, and the rationale for the approach were explained to the students at the start of the unit. Also, suggestions for ways in which the instructor can guide learning by asking open-ended questions, supporting and motivating students, and encouraging reflection to challenge further thinking

were built into the instructor's copy of the unit (Spronken-Smith et al., 2008). The rest of this chapter outlines specifics of the curriculum design as well as the study design, data collection, and data analysis methods.

Curriculum Design

This unit was developed with both the goal of incorporating inquiry-based instructional methods, as well as addressing topics typically found in an introductory postsecondary biotechnology class in order for the students to obtain college credit. Because the course is also designed to meet the students' high school graduation requirements, the unit was developed in accordance with *Next Generation Science Standards* (Achieve, Inc., 2013), National Healthcare Foundation Standards and Accountability Criteria (NCHSE, 2015), and Michigan Career and Technical Education Standards (Michigan Department of Education, 2009). Unit objectives, as developed by the researcher in collaboration with the instructor, are as follows:

1. Define the terminology related to molecular biology and use these concepts when discussing how molecular techniques may be used to understand genetic disorders or diseases.
2. Identify ways in which biotechnology is applied to various fields of research.
3. Perform agarose gel electrophoresis technique with minimal supervision and evaluate and interpret the results.

To introduce the students to the overall topic of biotechnology while engaging them in many of the Science and Engineering Practices outlined by the *NGSS*, the curriculum was broken into several lessons, to be taught over a three week period, that address the following topics:

DNA and chromosomal structure, genes, genetic traits and heredity, DNA isolation, polymerase chain reaction (PCR) amplification, agarose gel electrophoresis, human *Alu* PV92, genetic diseases and disorders, pharmacogenomics, and the analysis of scientific data, and communication of scientific information. Each lesson was designed to build upon both students' prior knowledge and the previous lesson by starting each class with a reflection of the prior day's topics and a student discussion of how they may apply to the lesson ahead. An outline of the lesson schedule is found in Table 3. Overall, the unit employs a variety of teaching strategies including laboratory investigations, classroom and small group discussion assignments, scholarly research, collaborative activities, and presentations.

Table 3. Lesson Schedule.

	Week 1
Monday	Lesson 1: Electrophoresis & Genetic Trait Exploration
Tuesday	Lesson 2: Dye Electrophoresis & Gene Exercises
Wednesday	Lesson 3: Class Discussion of Electrophoresis and Genetic Traits
Thursday	Lesson 4: Introduction to Human *Alu* PV92 & isolation of Cheek Cell DNA
Friday	Lesson 5: PCR Amplification of DNA
	Week 2
Monday	Lesson 6: DNA Analysis by Gel Electrophoresis
Tuesday	Lesson 7: Analysis and Interpretation of Your Results
Wednesday	Lesson 8: Molecular Techniques and Genetic Diseases/Disorders
Thursday	Lesson 9: Introduction to Genetic Based Diseases/Disorders
Friday	Lesson 10: Pharmacogenomics

Week 3

Recommendation: Give students 2-3 workdays so they have in-class time to work with their groups on the presentations.

Thursday	Lesson 11: Genetic Disorder/Disease Presentations – Day 1
Friday	Lesson 11: Genetic Disorder/Disease Presentations – Day 2 (if needed)

In this curriculum, student progression occurs from level one - confirmation to level three - guided inquiry activities. In the confirmation activities, the question, procedures or design, and results or analysis are all provided by the instructor. With guided-inquiry, the instructor still provides the question, but the rest of the investigation is directed by the student (Gengarelly & Abrams, 2009). The initial electrophoresis lesson and dye electrophoresis lab were conducted as level one inquiry, while the investigation into *Alu* PV92 was guided-inquiry. At the end of the unit, students were required to find a topic regarding a genetic disease or disorder that interested them and design a presentation using scholarly sources that ties in the concepts they had previously learned.

Each level of inquiry has its place in the science classroom. Since both students and educational goals are diverse, the form of inquiry used should depend largely on these factors (NRC, 2000). Structured, simple inquiry provides the basics of investigation to students and may be beneficial when new techniques or procedures need to be introduced for more complicated investigations to occur later. Guided inquiry may be more appropriate once procedures are familiar to students and there is flexibility regarding the outcome of the investigation. Open inquiry most closely resembles the work of actual scientists, but may depend on the ability of the teacher to facilitate student inquiry and the comfort of the student with the process (Zion &

Mendelovici, 2012). Since both the subject matter and teaching methods are new to both the students and the instructors, for the most part, it was decided that moving from level one to level three would best suit the educational goals of this unit (Fay and Bretz, 2008). This is also consistent with the goal of Science Teaching Standard B in the *National Science Education Standards*, to encourage the development of inquiry skills among students (NRC, 1996).

The alignment of the unit with various standards is summarized in Table 4 below. The unit objectives align with the *NGSS* performance standards HS-LS3 and HS-LS4. In the HS-LS3 standard of the *NGSS*, Heredity: Inheritance and Variation of Traits, it is stated that "technological advances have influenced the progress of science and science has influenced advances in technology" (p. 94). Also in the HS-LS4 standard, Biological Evolution: Unity and Diversity, it is stated that "genetic information provides evidence of evolution" and "adaptation also means that the distribution of traits in a population can change when conditions change" (p. 95). Students will examine both of these concepts through the connections made between biotechnology and human genetics and inheritance, as well as genetic disorders, in this unit. This curriculum addresses HS-LS3 by requiring students to investigate the role of DNA and chromosomes in coding for traits passed from parents to offspring, defend claims regarding causes for genetic variations, and apply statistics to explain variation. It also requires students to evaluate evidence regarding claims of environmental influences on population changes, construct an explanation of how natural selection leads to these changes, and communicate scientific evidence for these changes.

Students also engage in the eight practices of science and engineering that the *Framework* identifies as critical for student learning (NRC Framework, 2012). Students ask questions about genetic inheritance and their own genetic profile regarding *Alu* PV92. They

develop and use models for purposes of understanding genetic inheritance and genetic disease. Students plan for agarose gel electrophoresis and carry out the investigation, and analyze and interpret data, regarding their own genotype regarding *Alu* PV92. They use mathematics and computational thinking when assessing both the distribution of genotypes in their classroom population and when investigating their selected genetic disease. And, they obtain evidence, evaluate information, and communication details of their selected genetic disorders.

Students also engage in several crosscutting concepts through this unit (NRC Framework, 2012). Students observe patterns in nature and explore systems and system models related to inheritance and heredity through their exploration of human genetics. They explore cause and effect as they learn about both the impact of environmental and other factors on human genetics and the impact of various factors on the performance of agarose gel electrophoresis. Students also come to understand the importance of structure and function as they explore human genetics, the causes and effects of genetic disorders on human health, and the role of biotechnology in the diagnosis and treatment of these diseases.

This curriculum meets several outcomes of the National Healthcare Foundation Standards and Accountability Criteria. It addresses the academic foundation of human structure and function by helping the students realize the connections between structural of the human body at the molecular or genotypic level and functions at the phenotypic level. The unit also addresses medical mathematics by asking students to summarize genotypic and phenotypic results in terms of proportions compared to the larger population. It addresses the academic foundation of diseases and disorders by asking students to apply their knowledge of structure and function to situations in which structure goes awry, causing genetic diseases and disorders. Students are

required to research diagnosis, pathology, treatment, and prevention information as part of their final presentations.

During their research into genetic diseases and disorders, molecular techniques, and pharmacogenomics, students become acquainted with some of the ethical considerations that must be made with the advent of certain therapies. Their research on these therapies also introduces them to the impact emerging issues have on healthcare delivery systems and students practice both effective communication skills and key employability skills as they navigate teamwork, the development of their research, and the presentation of their disorder to the class.

This curriculum incorporates the technical standards, pathway standards, and career and employability standards from the Michigan Career and Technical Education Standards as well. The curriculum addresses the technical standards of academic foundations in human structure and function, diseases and disorders, and medical mathematics; concepts of effective communication; healthcare delivery systems; key employability skills, and ethics similarly to the National Healthcare Foundation Standards described previously. Additionally, this curriculum introduces students to personal and environmental safety through the practice of proper laboratory and specimen disposal protocol. The curriculum also incorporates information technology applications through the use of learning tools throughout the unit, either via videos or interactive websites, as well as encouraging students to use their resources during the exploration and research of genetic diseases and disorders.

The pathway standards again include the application of mathematical concepts by requiring student to calculate ratios to explain genetic variation. Students will increase their understanding of genetics through the construction of a karyotype and the exploration of commonly identifiable genetic traits and inheritance of such phenotypes. These concepts are then

applied to principles in the areas of biochemistry and molecular biology as students further explore DNA profiling through the agarose gel electrophoresis laboratory exercise.

This unit also meets the career and employability standards of the Michigan Career and Technical Education Standards by requiring students to apply academic skills such as reading and writing as they take the lead of their own learning using the tools provided in this unit. They are required to read for information and then apply that information in both the context of laboratory protocol and the presentation of information to others. The presentation specifically requires them to gather, interpret, analyze, and synthesize information and data from multiple sources and present this information using a variety of media. To accomplish all of this in the course of three weeks, along with their lab mates and presentation teammates, requires personal management, organization skills, and technology skills. These skills include but are not limited to regular attendance, staying on task, meeting deadlines, and adapting to changing circumstances, especially during a laboratory investigation.

Table 4. Alignment Between Educational Standards and Unit Lessons.

Standard	**Lesson**
Next General Science Standards High School (9-12) – Life Sciences	
HS-LS3 Heredity: Inheritance and Variation of Traits	
1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	1, 2, 3, 4, 7, 9
2. Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through	3, 4, 8, 9

meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.	
3. Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.	1, 2, 3, 7
HS-LS4 Biological Evolution: Unity and Diversity	
1. Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.	3, 4, 7, 11
4. Construct an explanation based on evidence for how natural selection leads to adaptation of populations.	4, 7, 9, 11
5. Evaluate the evidence supporting claims that changes in environmental conditions may result in: (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species.	3, 4, 7, 8, 9, 11
National Healthcare Foundation Standards and Accountability Criteria	
Foundation Standard 1: Academic Foundation	
1.1 Human Structure and Function	1, 2, 8, 9, 10
1.13 Analyze the interdependence of the basic structures and functions of the human body as they relate to wellness, disease, disorders, therapies, and care/rehabilitation.	1, 8, 9, 10
1.2 Diseases and Disorders	
1.22 Research common diseases and disorders of each body	

system (prevention, pathology, diagnosis, and treatment).	
1.23 Research biomedical therapies as they relate to the prevention, pathology, and treatment of disease.	1, 2, 7, 11
1.3 Medical Mathematics	
1.32 Analyze diagrams, charts, graphs, and tables to interpret healthcare results	
Foundation Standard 2: Communications	
2.1 Concepts of Effective Communication	3, 7, 11
2.13 Report subjective and objective information.	
Foundation Standard 3: Systems	
3.1 Healthcare Delivery Systems	8, 9, 10, 11
3.13 Assess the impact of emerging issues on healthcare delivery systems.	
Foundation Standard 4: Employability Skills	
4.2 Key Employability Skills	7, 8, 9, 10, 11
4.21 Apply employability skills in healthcare.	
Foundation Standard 6: Ethics	
6.1 Ethical Practice	7, 10, 11
6.12 Recognize ethical issues and their implications related to healthcare.	
Michigan Career & Technical Education Standards (26.0102 - Biotechnology Medical Sciences)	

Technical Standards		
I.	***Academic Foundation***	
A.	Human Structure and Function	1, 2, 3, 4, 7, 8, 9,
	3. Analyze the interdependence of the basic structures and functions of the human body as they relate to wellness, disease, disorders, therapies, and care/rehabilitation	10, 11
B.	Diseases and Disorders	1, 2, 8, 9, 10
	1. Compare diseases/disorders including respective classification(s), prevention, causes, pathogenesis, diagnoses, therapies, and care/rehabilitation	
	2. Investigate biomedical therapies as they relate to the prevention, pathology, and treatment of disease	
	3. Discuss complementary/alternative health practices as they relate to the prevention and treatment of disease	
C.	Medical Mathematics	1, 2, 7, 11
	1. Apply mathematical computations related to healthcare procedures	
	5. Analyze diagrams, charts, graphs, and tables to interpret healthcare results	
II.	***Communications***	
A.	Concepts of Effective Communication	3, 7, 11
	5. Report relevant information in order of occurrence	

	8. Report subjective and objective information	
	9. Use medical terminology to communicate information including data and observations	
C.	Written Communication Skills	3, 7, 8, 9, 10, 11
	2. Organize technical information and summaries	
III.	***Systems***	
A.	Healthcare Delivery Systems	3, 5, 6, 7, 8, 9,
	5. Explain the impact of 21st century emerging issues such as technology, epidemiology, bioethics, and socioeconomics on healthcare systems	10, 11
IV.	***Employability Skills***	
B.	Key Employability Skills	7, 8, 9, 10, 11
	2. Exemplify professional characteristics	
D.	Employability Preparation	3, 7, 11
	2. Execute work assignments and formulate solutions to problems using critical thinking skills	
VI.	***Ethics***	
A.	Legal and Ethical Boundaries	3, 8, 9, 10
	4. Recognize ethical issues and their implications related to healthcare	
VII.	***Safety Practices***	
B.	Personal Safety	4, 5, 6, 7
	2. Apply proper use of personal protective equipment	

	(PPE)	
C.	Environmental Safety 3. Understand proper safety techniques to prevent accidents and to maintain a safe work environment	4, 5, 6, 7
XI. Information Technology Applications		
A.	Health Information Management 4. Understand the content and diverse uses of health information	3, 7, 8, 9, 10
Pathway Standards		
II. Academic Foundations		
A.	Biotechnology R&D professional will be knowledgeable in the fundamentals of biochemistry, cell biology, genetics, mathematical concepts, microbiology, molecular biology, organic chemistry, and statistics 1. Apply Mathematical concepts a. Illustrate the concepts of percentages and ratios using a biotechnology application 3. Understand Genetics b. Construct a karyotype with human chromosomes c. Differentiate the genetic inheritance of a dominant homozygous trait (e.g. dwarfism) from a heterozygous (e.g. sickle cell anemia) 5. Apply principles of biochemistry	1, 2, 3, 4, 5, 6, 7, 8, 9, 10

	c. Describe the relationship between biochemistry and biotechnology product development	
	7. Apply principles of molecular biology	
	c. Describe the central dogma of molecular biology and how understanding this process impacts biotechnology research and development	
Career & Employability Standards		
I. Applied Academic Skills		
A.	Reading, English & Language Arts	3, 7, 11
	2. Give a verbal report on reading from a technical manual	
III. Developing and Presenting Information		
A.	Developing and Presenting information	3, 7, 11
	1. Gather, interpret, analyze, and refine data	
	2. Analyze and synthesize information and data from multiple sources	
	6. Practice and demonstrate presentation skills using a variety of media and interpretive data	
V. Personal Management		
A.	Responsibility	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
	1. Demonstrate regular attendance, promptness, and staying with a task until satisfactory completion	

	2. Complete assignments with minimum supervision and meet deadlines	
	3. Use mistakes as learning opportunities; demonstrate persistence and adaptability to change	
VI. Organizing Skills		
A.	Time	1, 2, 3, 4, 5, 6, 7,
	1. Determine goals and develop an action plan to accomplish them within a given time frame	8, 9, 10, 11
XI. Technology Skills		
B.	Social, Ethical, and Human Issues	3, 7, 10, 11
	8. Adhere to fair use and copyright guidelines	
	9. Create appropriate citations for resources when presenting research findings	
E.	Technology Research Tools	3, 8, 9, 10
	3. Determine if online sources are authoritative, valid, reliable, relevant, and comprehensive	
	4. Distinguish between fact, opinion, point of view, and inference	

Participants

This study employed convenience sampling, collecting data from students involved in the Kent Intermediate School District (KISD) Early College Program. The criteria for participation in this program were that each student was either a high school junior or senior within a Kent

County school district with a cumulative grade point average of 3.0 or higher. As the Early College Program was designed for students interested in science, specifically the fields of biology, biomedical science, and biotechnology, students were required to have successfully completed biology and were advised to also enroll in algebra I, anatomy and physiology, chemistry, and English I and II.

Data was collected in two phases. First, a pilot study was conducted using an initial curriculum design in February 2013 with one population of students. These data were used to determine where improvements were needed in the curriculum. After a redesign, the full study was conducted in November of 2014 with a second population of students. During both the pilot study and the full study, the sample population was composed of two classes (one morning and one afternoon) of high school seniors and juniors who elected to participate in the Kent County ISD Early College program.

The students meet each school day at a location in partnership with one of the local universities for approximately seventy-five minutes. The class is considered part of their high school curriculum and some dual college credit is available for some portions of the program. The program is coordinated and taught by an instructor employed through Kent ISD, but who is also recognized as adjunct faculty at this university. The instructor is certified in career and technical education and has a background in health sciences, but is a novice in inquiry-based instruction. The only portion of this unit used as an assessment for the students' regular coursework was the final presentation grade. The pre and posttest, student survey, and unit assignments were scored only to act as data for this study.

The participants in the full study totaled forty-six students between the two classes. Each student was given a participant number which acted as the identifier on all assignments and data

collection instruments for the researcher in order to protect actual student identities. Only the instructor knew the connection between student identity and participant number. Students self-selected groups for the collaborative work and final presentations.

Study Design

A pilot study was initially completed to ensure both successful implementation of a novel curriculum and effective data collection methods prior to performing the full study. It is also important to know that this unit was developed cooperatively between the classroom teacher and the researchers. As is characteristic of action research, the instructor participated in the process of planning the educational goals, conducting the research, grading of final presentations, and examining the ways in which the curriculum could be modified (Zion & Mendelovici, 2012).

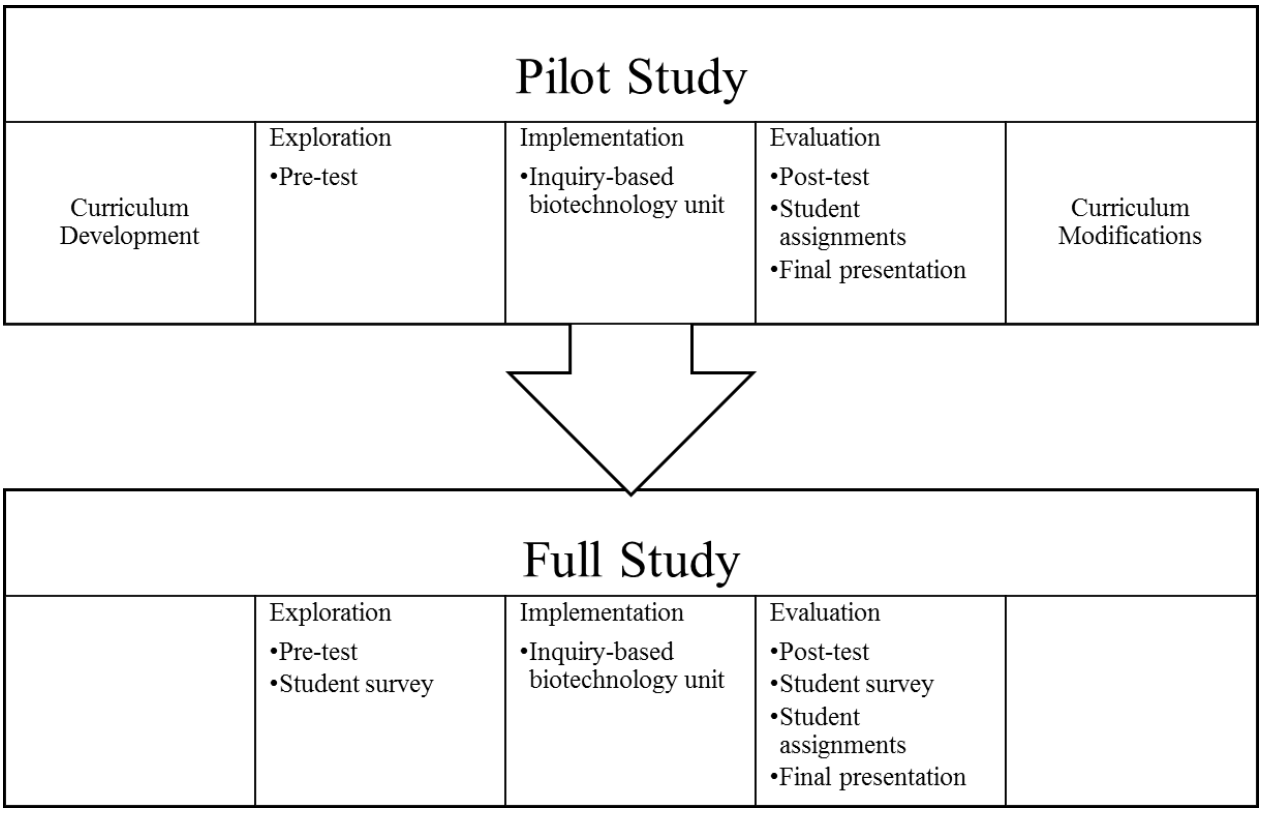


Figure 1. Framework of study design.

As shown in the figure above, the pilot phase included initial curriculum and assessment development. Once a cohort of students was identified, the appropriate pre-assessments were given in the exploration stage, the draft curriculum was implemented, and the appropriate post-assessments were given in the evaluation stage. Based on these assessments and observations made by both the instructor and researcher, adjustments were made to the curriculum and assessment instruments. These adjustments were made based on the need for improved learning outcomes as well as stronger efficacy in the area of inquiry-based instruction. Once the next cohort of students was identified, the full study commenced. Again, pre-assessments were given during the exploration stage, the curriculum was implemented, and the post-assessments were given during the evaluation stage.

The study used a quasi-experimental design conducted using a one-group pretest-posttest, or repeated measures (Harris *et al*., 2006). Because the same students took both the pre-assessments and post-assessments, the study qualifies, specifically, as a within-participant design. This design was chosen primarily for ethical considerations. Several threats to validity have been identified for this type of design; internal validity, statistical conclusion validity, and construct validity (Duckart, 1998). However, measures were taken to reduce these threats. Regarding internal validity, because maturation is often a concern, time between pre-test, intervention, and post-test was reduced to only two days each. History was not a concern because there were no other interventions regarding either the curriculum or the form of instruction at the time of this study. Concerns with testing were minimal as well because no feedback was given to the students after the pre-assessments were administered. And, attrition was not significant. Concerns regarding statistical conclusion validity will be reduced by the appropriate selection of statistical analysis and recognition of the limitations and delimitations. Variability in treatment

implementation is not a concern because the curriculum was implemented by the same person during the entire study to all students.

Data Collection

This study was primarily quantitative and assessed both student content knowledge and biotechnology process learning outcomes. Student motivation towards learning science was also addressed quantitatively via a Likert-type assessment and overall student opinion of both the instructor and the course were assessed to some extent using qualitative open-ended survey questions. Data were collected from the following sources:

1) Pre and posttests;

2) Pre and post-assessments;

3) Biotechnology unit assignments and final student presentations, and

4) Student surveys.

The pre and posttest, student assignments, and the final presentations provide measures of content knowledge and biotechnology process learning outcomes. The pre and posttest was a seventeen question, multiple choice evaluation that was developed by the researcher who adapted questions from several sources that included the 1999 released exam for Advanced Placement (AP) Biology (The College Board, 1999), test questions from *Chapter 1: Overview of Genetics* from McGraw-Hill Companies' *Human Genetics: Concepts and Applications, 9th edition* (Lewis, 2009), and test questions from *Chapter 20: Biotechnology* from Pearson Education's *Campbell Biology, 9th edition* (Reece, Taylor, Simon, & Dickey, 2010). Face validity and curricular validity were assessed by both the researcher and the course instructor who agreed test questions were in alignment with learning outcomes. Questions one through five address the area of human genetic traits and heredity (Lewis, 2009), questions six and seven

(Lewis, 2009 and Reece et al., 2010, respectively) address genetic variation and mutation, questions eight and nine (Reece et al., 2010) address polymerase chain reaction, questions ten through twelve (The College Board, 1999) address gel electrophoresis, and questions thirteen through fifteen (Lewis, 2009) address genetic disorders and DNA profiling.

The final presentations were evaluated using a rubric adapted by the researcher from the *Rubric for Oral Presentations* (New England Association of School and Colleges, 2016) (Table 5). The rubric included six areas of evaluation: subject knowledge, organization and coherence; physical presentation; language convention; visual aids; and scholarly sources. The rubric was divided into 'exceeds standards', 'meets standards', 'emerging', and 'attempt made' for each section. Total points for each area were 60, 5, 10, 5, 10, and 10, respectively, leading to a maximum overall score of 100 points (Table 5).

The oral presentation speaks to the learning objectives by providing students an opportunity identify ways in which biotechnology is applied to various fields during the research required for the presentation. It also provides students with an opportunity to define and appropriately use the terminology related to molecular biology when discussing how molecular techniques may be used to understand genetic disorders and diseases. The presentation also addresses the *Next Generation Science Standards* in the area of heredity; the National Healthcare Foundation Standards and Accountability Criteria in the areas of human structure and function, diseases and disorders, and concepts of effective communication; and the Michigan Career and Technical Education Standards in the areas of human structure and function, diseases and disorders, concepts of effective communication, and the broader areas of developing and presenting information, personal management, organizing skills, and technology skills.

Table 5. Presentation Grading Rubric.

	Exceeds Standard	Meets Standard	Emerging	Attempt Made
Subject Knowledge	Demonstrates mastery of the topic in each of the following categories; what the disease is, how people get it, symptoms, diagnosis, treatment, and interesting facts.	Demonstrates accurate knowledge of the topic in each of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment	Demonstrates only some knowledge of the topic, and is missing one or two of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment	Demonstrates little knowledge of the topic, and is missing more than three of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment
Organization and Coherence	Organizes information coherently, stays on the topic	Organizes most information, stays on the topic	Generally organizes information, occasionally strays from the topic	Poorly organizes information, often strays from the topic
Physical Presentation	Always speaks clearly/loudly, actively engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Usually speaks clearly/loudly, usually engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Speaks clearly/loudly, occasionally engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Does not speak clearly/loudly, neglects to engage the audience, rarely makes and maintains eye contact or uses movement to focus attention/interest
Language Convention	Uses appropriate grammar and vocabulary	Mostly uses appropriate grammar and vocabulary	Makes some errors in grammar and vocabulary	Makes many mistakes in grammar and vocabulary
Visual Aids	Creatively uses a variety of effective visual aids and/or other methods of delivery	Uses visual aids moderately effectively and/or other methods of delivery	Moderately ineffective use of some visual aids and/or other methods of delivery	Does not use of visual aids and/or other methods of delivery
Scholarly Sources	More than two additional sources included and appropriately cited	Two additional scholarly sources included and appropriately cited	Either only one additional scholarly source included or not appropriately cited	Lacking two additional scholarly sources and not appropriately cited

Table 6. Presentation Evaluation Form.

	Exceeds Standard	Meets Standard	Emerging	Attempt Made
Subject Knowledge	60	50	40	30
Organization & Coherence	5	4	3	2
Physical Presentation	10	8	6	4
Language Convention	5	4	3	2
Visual Aids	10	8	6	4
Scholarly Sources	10	8	6	4

The biotechnology unit assignments provided formative assessments along the way to gauge student learning in comparison to the other evaluations. These were each scored on their own point system as established by the researcher.

The student assessment, Students' Motivation Toward Science Learning, used to measure motivation, was taken from Tuan, Chin, and Shieh (2005). This questionnaire evaluated six areas of motivation:

1. Self-efficacy: students' belief in their ability to perform well in science,
2. Active learning strategies: students taking an active role in using many methods to construct new understanding from previous knowledge,
3. Science learning value: student motivation for learning science because they perceive value in that learning,
4. Performance goal: the student's goal in learning science is to perform better than other students,
5. Achievement goal: student satisfaction corresponds to improved ability for science learning, and

6. Learning environment stimulation: the classroom environment, including content, motivates student science learning.

Taken together, this assessment is a measure of the affective component of cognition. Researchers have begun to stress the importance of the affective components of learning (Duit & Treagust, 1998; Lee, 1989; Lee & Brophy, 1996; Pintrich *et al*., 1993; Strike & Posner, 1983 & 1992; West & Pines, 1983). Within these affective components, motivation in particular is important in the development of critical thinking skills, learning strategies, and science learning achievement (Garcia & Pintrich, 1992; Kuyper *et al*., 2000; Napier & Riley, 1985; Wolters, 1999). Since previous research has shown that inquiry-based instruction has been successful in increasing student motivation for learning in both science generally and biotechnology specifically (Klop et al., 2010; Movahedzadeh, Patwell, Rieker, & Gonzalez, 2012; Taraban, Box, Myers, Pollard, & Bowen, 2007; Lesmes Celorrio, Fernandez Gomez-Chacon, & Gonzalez-Soltero, 2013), the decision was made to measure this component of this newly developed curriculum as well. This specific assessment was selected because it assesses student motivation toward learning science while many others assess a more generalized nature of motivation toward learning.

Questionnaire reliability was tested by selecting 15 senior high schools in central Taiwan and randomly selecting one class from each grade from each school to complete the questionnaire ($n = 210$). The Cronbach alpha for the overall questionnaire was 0.89, while for each scale it ranges from 0.70 to 0.89. The questionnaire has been shown to have significant ($p < 0.01$) correlations with students' science attitudes ($r = 0.41$), and there was a significant difference between "high motivators" and "low motivators" ($p < 0.01$). The 35 statements were

evaluated using a five-point Likert-type scale (1 = strongly disagree, 2 = disagree, 3 = no opinion, 4 = agree, and 5 = strongly agree).

Data Analysis

The pretest and posttest addressed the first research question: "What are the effects of an inquiry-based biotechnology unit on students' content knowledge?" Regarding the pretest/posttest, the null hypothesis was that there would be no change in content knowledge after students experienced an inquiry-based biotechnology curriculum. The alternative hypothesis was that there would be a (positive) change in content knowledge.

$$H_0: \mu_D = 0$$

$$H_1: \mu_D > 0$$

Raw scores were converted to percentages, and data was analyzed using IBM SPSS Statistics 20 software. After normality was confirmed using the Shapiro-Wilk test and assessing Q-Q plots, a paired samples *t*-test, consistent with one group pretest-posttest design, was use to compare pre and posttest scores. A ninety-five percent confidence interval and a Cohen's d value were calculated as well.

To answer the second research question, "How do students' perceptions of their learning differ between pre- and post-assessment after engaging in an inquiry-based biotechnology unit?", the student survey was used. Again, the null hypothesis was that there would be no change, while the alternative was that there would be a change in student perception toward inquiry-based biotechnology education.

$$H_0: \mu_D = 0$$

$$H_1: \mu_D > 0$$

The responses from the Likert-scale survey questions were also converted from their raw scores to percentages and each subscale, as well as the whole scale, were assessed for their intraclass correlation coefficient and Cronbach's alpha was calculated for each in order to determine internal consistency. The scales were then tested for normality, again using the Shapiro-Wilk test and Q-Q plots. Those scales that were found to be normal were assessed using a paired samples *t*-test to compare pre and posttest scores and a ninety-five percent confidence interval was calculated for each. There is debate as to whether or not parametric tests are appropriate for Likert-scale data, given the ordinal data. However, leading researchers in medical education research methodology, such as Dr. Geoff Norman, do support the use of parametric tests, and support parametric tests as being more robust compared to nonparametric tests (Sullivan & Artino, 2013). For the remaining scales that were not found to be normal, data was evaluated using the Wilcoxon-signed rank test.

The interaction between the biotechnology curriculum and both content knowledge and student motivation was evaluated in an effort to tie the two research questions together. By performing path analysis via multiple regression modeling, a holistic view of the study was taken to determine how the variables interrelate and effect both student content gains and student motivation toward their learning together. For this analysis, the null hypothesis was that there would be no relationship between the included variables, while the alternative hypothesis was that there would be some relationship between at least two of the variables. Student assignments and final presentation raw scores were converted to percentages and a hypothesized full model was developed based on identified criterion and predictor variables. Three layers of path analysis were determined and each was assessed by first by ANOVA and then multiple regression to determine if any relationship existed between variables. Based on these analyses, a reduced

model of path analysis could be developed that included those significant relationships between the variables of student learning.

For all analyses related to this study, $p < .05$ will be considered significant. Open-ended survey questions provided a qualitative component to the question regarding student motivation and served to supplement the quantitative data captured by this study. .

Ethical Considerations

The University Institutional Review Board (IRB) has determined this study is covered human subjects research according to the current federal regulations and that it meets eligibility for exempt determination under category 45 CFR 46.101(b)(1) (GVSU HRRC Reference number 14-097-H). It meets this regulation because this research aims to study the effectiveness of a curriculum that is being implemented using regular educational practices in a commonly accepted educational setting. In addition to the exempt status, students were fully informed of the purpose of the study and were provided the contact information for the researcher. The researcher also verbally shared her background knowledge in relation to the research topic and explained specifically what portions of the unit would be graded as part of the students' regular course. The researcher ensured all participants were given the necessary information about the research, consent, and confidentiality and explained there were no known risks to participation in the study. The researcher explained that no identifying student information, nor school information, would be included in the study, and that each student would only be known by a participant number to the researcher and in the data collected. The researcher also ensured the students that a final copy of the research would be made available to them through their course instructor should they want to view it once it as completed. The exempt approval memo has been included in Appendix A.

Chapter IV – Results

The purpose of this study was two-fold; to meet the need of a local Early College program for a hands-on, inquiry-based biotechnology curriculum, and to evaluate the impact of such an educational program on students' learning outcomes and their perceptions of science learning. Inquiry-based learning is a pedagogy through which the instructor acts as a facilitator, supporting students in the construction of knowledge as they pose questions, engage in investigation of problems or scenarios, and construct explanations based on these investigations (Khalid & Azeem, 2012). Biotechnology education is the study of the creation of products beneficial to humans through the use of organisms or parts of organisms (Bigler and Hanegan, 2011). This study focused on the unique intersection of this field and pedagogy and assessed the impact of an inquiry-based biotechnology unit on both students' content knowledge and perceptions of learning via a quantitative, quasi-experimental design. All quantitative data was analyzed using IBM SPSS Statistics Version 20 software.

Sample

As described in Chapter III, student participants in this study were enrolled in the Kent Intermediate School District (KISD) Early College Program. Participants in the full study, the results of which are described in this chapter, were enrolled in the 2014-15 cohort. Demographic information of the sample, including class status, gender, ethnicity, and school environment, is summarized in Tables 7-10 below.

Table 7. Student participant school grade descriptive statistics.

	Frequency	Percent	Valid Percent	Cumulative Percent
Junior	33	71.7	71.7	71.7
Senior	13	28.3	28.3	100.0
Total	46	100.0	100.0	

Table 8. Student participant sex descriptive statistics.

	Frequency	Percent	Valid Percent	Cumulative Percent
Female	32	69.6	69.6	69.6
Male	14	30.4	30.4	100.0
Total	46	100.0	100.0	

Table 9. Student participant school environment descriptive statistics.

	Frequency	Percent	Valid Percent	Cumulative Percent
Rural	10	21.7	21.7	21.7
Suburban	16	34.8	34.8	56.5
Urban	20	43.5	43.5	100.0
Total	46	100.0	100.0	

Table 10. Student participant ethnicity distribution.

	Frequency	Percent	Valid Percent	Cumulative Percent
African American	7	15.2	15.2	15.2
Asian	1	2.2	2.2	17.4
Caucasian	24	52.2	52.2	69.6
Hispanic	10	21.7	21.7	91.3
Multi-Racial	2	4.3	4.3	95.7
Not Reported	2	4.3	4.3	100.0
Total	46	100.0	100.0	

Student Content Knowledge

In addressing the first research question, "What are the effects of an inquiry-based biotechnology unit on students' content knowledge?" a content test consisting of seventeen multiple choice questions was administered as a pre and posttest (Appendix B). The pretest was administered approximately one week prior to the start of the study and the posttest was given one week after the end of the study. Both tests were administered in paper and pencil format and the students were given ample time to complete the assessment. The samples size, defined as students completing both the pretest and posttest, was 45. Scores were converted to percentages by dividing the number of correct responses by seventeen and the difference [posttest – pretest] was calculated for each student. If a student did not get any answers correct, the score and corresponding percentage were recorded as zero. If the student did not take the test, the data point was recorded as missing. The pairwise differences ranged from a minimum of -23.53 to a maximum of 64.71 with a mean of 13.99 and standard deviation of 16.75. The data were checked

for normality using the Shapiro-Wilk test (Table 11) and normal Q-Q plot (Figure 2). Since the Shapiro-Wilk test is significant (p = 0.366), and the data points are closely associated with the line in the normal Q-Q plot, it can be assumed that the data are normally distributed.

Table 11. Shapiro-Wilk test for normality of student pre- and posttest data.

	Shapiro-Wilk		
	Statistic	df	Sig.
Pairwise Differences	.973	45	.366

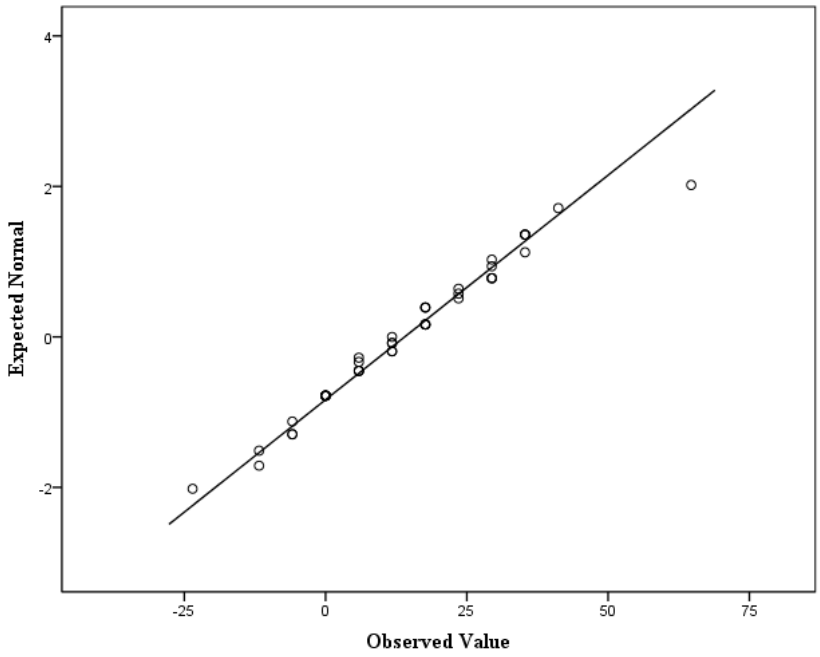


Figure 2. Normal Q-Q plot test for normality of student pre- and posttest data.

Since normality was confirmed, a paired samples *t*-test was performed, which indicated the scores were significantly higher on the posttest (M = 66.4, SD = 14.4) compared to the pretest (M = 52.4, SD = 16.8), $t(44) = 5.603$, $p < .000$, $d = 0.835$. The researcher can be ninety-five percent confident (Table 12) that student content knowledge increased by between 8.96 and 19.02 percentage points from pretest to posttest ($p < .000$). The effect size, or Cohen's d value, for this assessment is deemed as a large treatment effect.

Table 12. Paired samples *t*-test for student pre- and posttest data.

			95% Confidence Interval of the Difference				
Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
13.98693	16.74564	2.49629	8.95598	19.01788	5.603	44	.000

Students' Motivation Toward Science Learning

To address the second research question, "How do students' motivation toward science learning differ between pre- and post-assessment after engaging in an inquiry-based biotechnology unit?" the Student Motivation Towards Learning (SMTSL) (Appendix B) was administered to the students as a pre and post-assessment. Again, the pre-assessment was given approximately one week prior to the start of the study and the post-assessment was given one week after the end of the study. The assessments were administered in paper and pencil format and the students were given ample time to complete the survey. The assessment is composed of six subscales in the areas of self-efficacy, active learning strategies, science learning value,

performance goal, achievement goal, and learning environment stimulation and together they measure the affective component of cognition specifically in the area of science learning. The assessment results were recorded in their raw form (i.e. Likert scale value 1-5). Reverse items (questions 2, 4, 5, 6, 7, 21, 22, 23, and 24) were recoded so that responses of 1 became 5, 2 became 4, 3 remained 3, 4 became 2, and 5 became 1. Subscale scores were then found by summing the scores of individual questions within each scale (Table 13).

Table 13. Subscale question composition within the students' motivation towards science learning questionnaire.

Subscale	Questions	Total Items
Self-efficacy	1, 2, 3, 4, 5, 6, 7	7
Active learning strategies	8, 9, 10, 11, 12, 13, 14, 15	8
Science learning value	16, 17, 18, 19, 20	5
Performance goal	21, 22, 23, 24	4
Achievement goal	25, 26, 27, 28, 29	5
Learning environment stimulation	30, 31, 32, 33, 34, 35	6

Table 14. Subscale consistency indicators for both pre-assessment and post-assessment.

Subscale		N	Cronbach's Alpha	95% Confidence Interval		F Test		
				Lower Bound	Upper Bound	Value	df	Sig.
Self-efficacy	Pre	44	.829	.739	.896	5.843	43	.000
	Post	46	.866	.797	.917	7.444	45	.000
Active learning	Pre	44	.851	.773	.909	6.701	43	.000
strategies	Post	45	.854	.779	.910	6.826	44	.000
Science learning value	Pre	43	.777	.650	.867	4.478	42	.000
	Post	46	.866	.793	.919	7.459	45	.000
Performance goal	Pre	43	.840	.745	.906	6.250	42	.000
	Post	46	.880	.811	.928	8.320	45	.000
Achievement goal	Pre	43	.746	.602	.849	3.937	42	.000
	Post	46	.838	.750	.902	6.175	45	.000
Learning environment	Pre	44	.738	.597	.842	3.815	43	.000
stimulation	Post	46	.858	.783	.913	7.034	45	.000
SMTSL	Pre	43	.903	.856	.940	10.286	42	.000
	Post	45	.874	.815	.921	7.939	44	.000

The intraclass correlation coefficient (ICC) was assessed for each subscale for both the pre-assessment and post-assessment as well as the overall questionnaire (Table 14). Because Cronbach alpha for all subscales and the overall questionnaire, both in the pre-assessment and

post-assessment, are greater than 0.70 and the F values are all significant ($p < .000$), the scales are determined to be internally consistent.

Subscale totals and the overall total were converted to percentages by dividing the sum of the scales by the total score possible (number of items multiplied by 5). Pairwise differences were then found by subtracting pre scores for each subscale and the overall total from their respective post score values. The pairwise difference data were checked for normality using the Shapiro-Wilk test (Table 15) and normal Q-Q plot (Figure 3). Since the Shapiro-Wilk test is significant ($p > 0.05$) and the data points are closely associated with the line in the normal Q-Q plot for some subscales, it can only be assumed that the data are normally distributed for the overall total and the following subscales; active learning strategies, performance goal, and achievement goal.

Table 15. Shapiro-Wilk test for normality of student pre- and post-assessment data.

	Shapiro-Wilk		
Subscale	Statistic	df	Sig.
Self-efficacy	.939	44	.022
Active learning strategies	.975	43	.461
Science learning value	.912	43	.003
Performance goal	.969	43	.281
Achievement goal	.973	43	.392
Learning environment stimulation	.937	44	.019
SMTSL	.975	42	.490

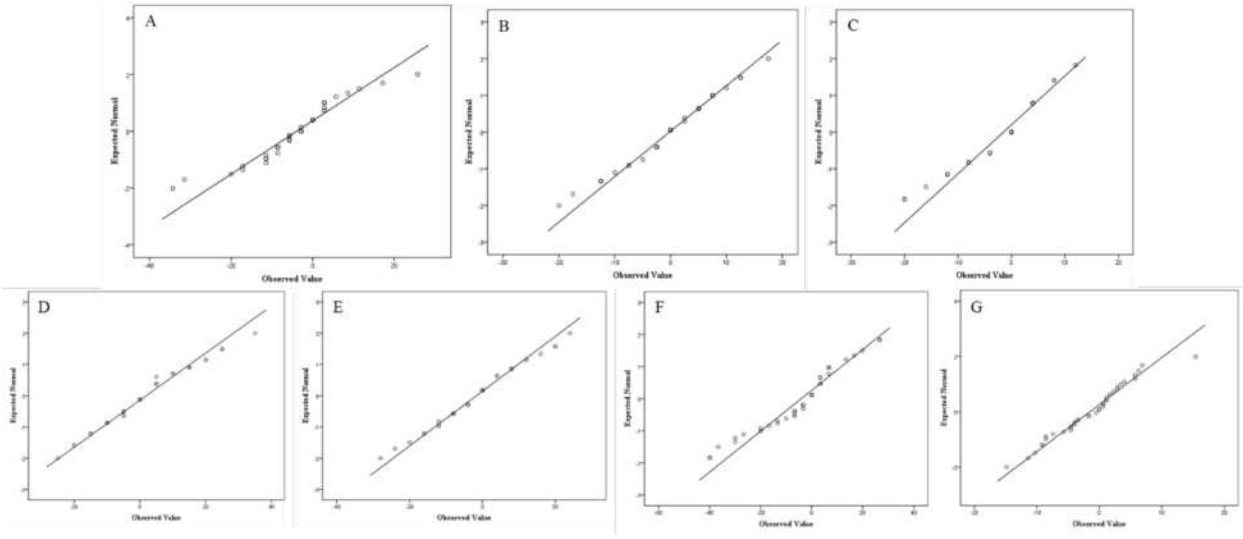


Figure 3. Normal Q-Q plot test for normality of student pre- and post-assessment data. Scales are designated as follows; A) self-efficacy, B) active learning strategies, C) science learning value, D) performance goal, E) achievement goal, F) learning environment stimulation, and G) total scale.

Table 16. Paired samples *t*-test for student pre- and post-assessment active learning strategies data.

			95% Confidence Interval of the Difference				
Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
-.23256	8.06989	1.23065	-2.71611	2.25099	-.189	42	.851

Table 17. Paired samples *t*-test for student pre- and post-assessment performance goal data.

			95% Confidence Interval of the Difference				
Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
1.86047	13.27472	2.02438	-2.22489	5.94582	.919	42	.363

Table 18. Paired samples *t*-test for student pre- and post-assessment achievement goal data.

			95% Confidence Interval of the Difference				
Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
-1.67442	11.42204	1.74184	-5.18960	1.84077	-.961	42	.342

Table 19. Paired samples *t*-test for student pre- and post-assessment total scale data.

			95% Confidence Interval of the Difference				
Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
-1.53741	5.88658	.90832	-3.37180	.29697	-1.693	41	.098

Since normality could be confirmed for the overall instrument total and the active learning strategies, performance goal, and achievement goal subscales, paired sample *t*-tests were conducted of these data. None of the tests retuned significant findings, suggesting there was no detectable change in these scales regarding students' motivation toward science learning due to the treatment (Tables 16-19).

Since the remaining subscales (self-efficacy, science learning value, and learning environment stimulation) did not meet conditions of normality, the nonparametric Wilcoxon Signed-Rank test was performed in each case. For self-efficacy, pre-assessment scores were higher (*Mdn* = 30) than post-assessment scores (*Mdn* = 28), and the Wilcoxon-signed rank test (Table 20) resulted in a statistically significant decrease from pre- to post-assessment (Z = -2.677, p = .007). For both the science learning value (*pre-Mdn* = 22, *post-Mdn* = 21) and the learning environment stimulation (*pre-Mdn* = 22, *post-Mdn* = 20.5) subscales, pre-assessment scores were higher than post-assessment scores, but the Wilcoxon-signed rank tests (Tables 21-22) did not result in a statistically significant decrease (Z = -1.135, p = .256 and Z = -1.444, p = .149, respectively).

Table 20. Wilcoxon-signed rank for student pre- and post-assessment self-efficacy data.

	N	Mean Rank	Sum of Ranks	Z	Asymp. Sig. (2-tailed)
Negative Ranks	25[a]	20.10	502.50	-2.677	.007
Positive Ranks	11[b]	14.86	163.50		
Ties	8[c]				
Total	44				

a. Post A Subscores < Pre A Subscores
b. Post A Subscores > Pre A Subscores
c. Post A Subscores = Pre A Subscores

Table 21. Wilcoxon-signed rank for student pre- and post-assessment science learning value data.

	N	Mean Rank	Sum of Ranks	Z	Asymp. Sig. (2-tailed)
Negative Ranks	14[a]	18.00	252.00	-1.135	.256
Positive Ranks	14[b]	11.00	154.00		
Ties	15[c]				
Total	43				

a. Post C Subscores < Pre C Subscores
b. Post C Subscores > Pre C Subscores
c. Post C Subscores = Pre C Subscores

Table 22. Wilcoxon-signed rank for student pre- and post-assessment learning environment stimulation data.

	N	Mean Rank	Sum of Ranks	Z	Asymp. Sig. (2-tailed)
Negative Ranks	20[a]	21.23	424.50	-1.444	.149
Positive Ranks	16[b]	15.09	241.50		
Ties	8[c]				
Total	44				

a. Post F Subscores < Pre F Subscores
b. Post F Subscores > Pre F Subscores
c. Post F Subscores = Pre F Subscores

In summary, looking at students' perceptions of their learning alone, results show that, for the most part, students' perceptions of their learning did not measurably change between pre- and post-assessment, except for in the case of self-efficacy where subscale scores decreased from pre- to post-assessment.

Interaction between Student Content Knowledge, Students' Motivation Toward Science Learning, and Biotechnology Curriculum

To further investigate the impact an inquiry-based biotechnology unit might have on student content knowledge and students' perceptions of their learning, additional analyses were performed that would take into consideration student performance during the unit by considering student assignment and final presentation scores. To do this a simple path analysis via regression was employed.

To prepare the newly included student assignment and final presentation score data, raw student scores, based on grading rubrics for the given assignments and final presentation, were converted to score percentages. Again, if a student attempted an assignment, but did not get any correct answers, the assignment is scored as a zero. If a student was not present or did not attempt the assignment for any other reason, the assignment is recorded as a missing data point. An assignment average was then calculated for each student by taking the average across the assignments each student completed. This assignment average data was then combined into a master file along with pre-assessment and post-assessment percentages for students' perceptions of their science learning and pretest and posttest percentages for students' content knowledge.

The hypothesized path analysis includes the possibility that all variables;

- pretest (Pre_Test_Perc),
- pre-assessment (Pre_Mot_Perc),
- assignment averages (Assign_Ave),
- post-assessment (Post_Mot_Perc), and
- posttest (Post_Test_Perc),

are interrelated and have an impact on the outcomes of each other. This is modeled in the Figure 4 below in which arrows designated predictor or causal variables and dependent or criterion variables. For analysis of the full model, three layers of multiple regressions will be needed:

1. Assign_Ave as the criterion and Pre_Test_Perc and Pre_Mot_Perc as the predictors,
2. Post_Mot_Perc as the criterion and Assign_Ave and Pre_Mot_Perc as the predictors, and
3. Post_Test_Perc as the criterion and Pre_Test_Perc, Assign_Ave, and Post_Mot_Perc and the predictors.

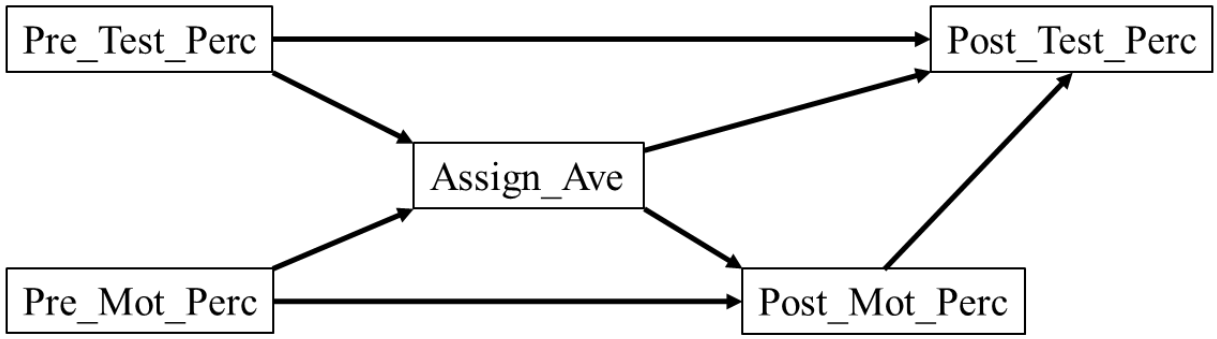


Figure 4. Hypothesized full model simple path analysis.

Table 23. ANOVA for first layer path analysis, dependent variable Assign_Ave.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	95.458	2	47.729	.749	.480
Residual	2550.146	40	63.754		
Total	2645.604	42			

Table 24. Multiple regression for first layer path analysis, dependent variable Assign_Ave.

	Unstandardized Coefficients		Standardized Coefficients				R
Model	B	Std. Error	Beta	t	Sig.	R	Square
(Constant)	.000	1.218		.000	1.000	.190	.036
Pre_Test_Perc	-.006	.075	-.012	-.077	.939		
Pre_Mot_Perc	-.186	.158	-.187	-1.183	.244		

Table 25. ANOVA for second layer path analysis, dependent variable Post_Mot_Perc.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	1440.981	2	720.490	23.335	.000
Residual	1235.064	40	30.877		
Total	2676.045	42			

Table 26. Multiple regression of second layer path analysis, dependent variable Post_Mot_Perc.

	Unstandardized Coefficients		Standardized Coefficients				R
Model	B	Std. Error	Beta	t	Sig.	R	Square
(Constant)	.000	.847		.000	1.000	.734	.538
Assign_Ave	-.088	.110	-.087	-.797	.430		
Pre_Mot_Perc	.713	.110	.712	6.511	.000		

Table 27. ANOVA for third layer path analysis, dependent variable Post_Test_Perc.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	3129.465	3	1043.155	7.551	.000
Residual	5388.067	39	138.156		
Total	8517.5532	42			

Table 28. Multiple regression of third layer path analysis, dependent variable Post_Test_Perc.

	Unstandardized Coefficients		Standardized Coefficients				R
Model	B	Std. Error	Beta	t	Sig.	R	Square
(Constant)	.000	1.792		.000	1.000	.606	.367
Pre_Test_Perc	.289	.114	.341	2.541	.015		
Assign_Ave	.634	.234	.353	2.704	.010		
Post_Mot_Perc	.612	.245	.343	2.491	.017		

Path coefficients were taken as the β weights from the multiple regression analyses above (Tables 24, 26, 28), and *e* values (roughly error of variance) were computed as $\sqrt{(1-R^2)}$ from those same tables. These values can be found in the updated full model in Figure 5.

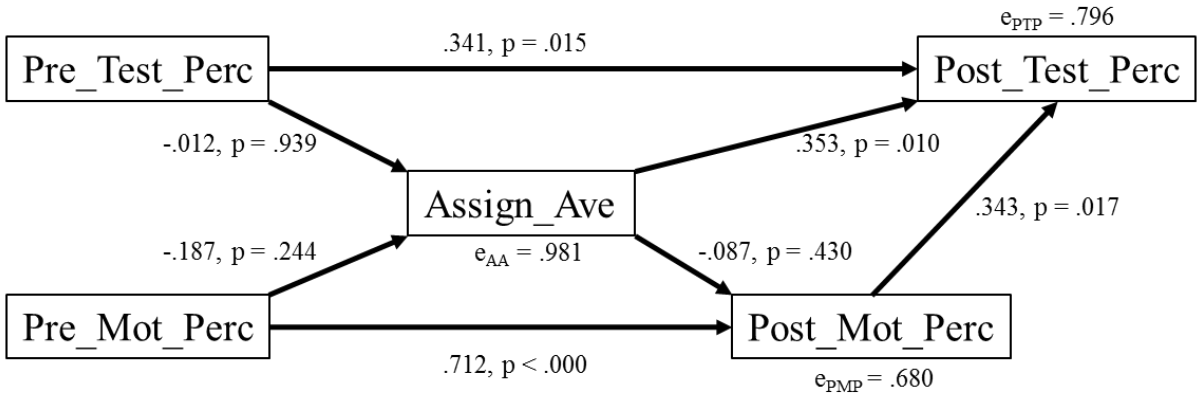


Figure 5. Full model simple path analysis with regression analysis results.

Examining this model, the results indicate:

1. Pre_Test_Perc, Assign_Ave, and Post_Mot_Perc directly influence Post_Test_Perc.
2. While Assign_Ave does not influence Post_Mot_Perc, Pre_Mot_Perc does and it indirectly influences Post_Test_Perc through Post_Mot_Perc.
3. Neither Pre_Test_Perc nor Pre_Mot_Perc influences Assign_Ave.

These conclusions result in the reduced path analysis model below (Figure 6).

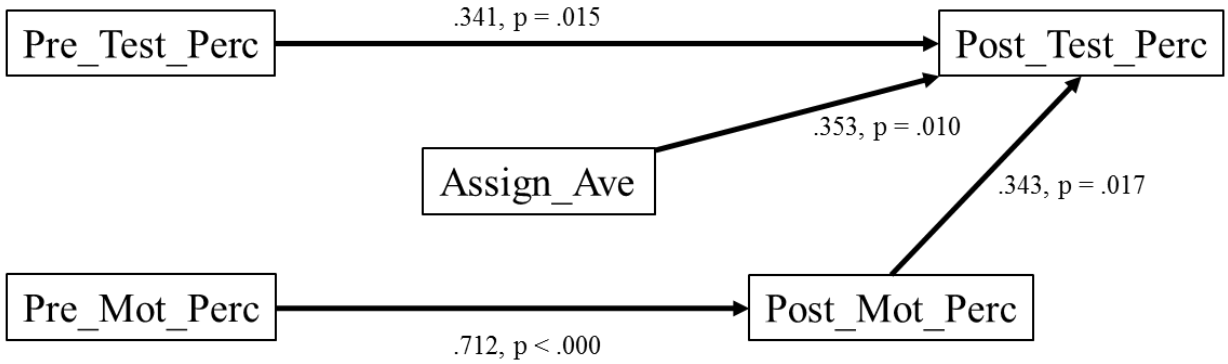


Figure 6. Reduced model simple path analysis with statistically significant regression analysis results.

Summary

These data indicate, when controlling for student assignments and the final presentation, students' motivation toward science learning post-assessment increased by 0.713 percentage points for every one percentage point increase in their pre-assessment ($F = 23.335$, $p < .000$). They also show that student content knowledge increased as a function of previous content knowledge, student assignments and the final presentation, and concluding students' motivation toward science learning as modeled by the following equation ($F = 7.551$, $p < .000$):

$$Post\_Test\_Perc = 0.289(Pre\_Test\_Perc) + 0.634(Assign\_Ave) + 0.612(Post\_Mot\_Perc)$$

Chapter V – Discussion

Introduction

This study provides an important link between biotechnology education and inquiry-based instruction. Biotechnology is becoming increasingly integrated into our lives from personal to political matters (Hengan and Bigler, 2009). However, although the memorization of facts was the prevalent model of education throughout the twentieth century, developing scientific literacy in biotechnology requires more than the traditional dissemination of facts from teacher to student, demanding a new model of education that will meet the changing needs of our society (Friesen, 2009). Inquiry-based learning is a student-centered model built on the theoretical framework of constructivism, whereby the learner constructs knowledge through the interaction between experiences and ideas (Duffy & Cunningham, 1996). Inquiry allows students to learn in a way that reflects how scientists come to understand the natural world.

Despite their growing significance, both biotechnology and inquiry-based instruction has not been widely adopted in science curricula (Bigler & Hanegan, 2011; Borgerding, Sadler, & Koroly, 2013). Educators cite various challenges to implementing both inquiry-based instruction and biotechnology education in their classrooms including accessibility of investigation techniques and management of extended activities, and comfort with the content knowledge and equipment (Borgerding, Sadler, & Koroly, 2013; Edelson, Gordin, & Pea, 1999). For this reason, this study focused on the unique intersection of biotechnology education and inquiry-based pedagogy.

The purpose of the study was to address the need for a hands-on, inquiry-based curriculum that will introduce biotechnology into the teaching of introductory and foundational biology concepts to meet the specific needs of a local Early College program, while

simultaneously evaluating the impact of this inquiry-based biotechnology curriculum on student learning outcomes and students' motivation toward their science learning. Because there is a lack of adoption of both inquiry-based teaching and learning (Crawford, 2007; Zion & Mendelovici, 2012) and biotechnology education (Bigler & Hanegan, 2011; Borgerding, Sadler, & Koroly, 2013) within the science curriculum, this study aimed to add to the literature and fill this gap in the curriculum by providing a case study of inquiry-based biotechnology education.

Discussion of Findings

Limitations. As with all educational research studies there are certain limitation to this study which impact the generalizability of the findings. Due to the nature of this study implementation was limited to once per year in order to have the material fall within the normal curriculum cycle of the participating Early College program. The newly developed materials were first introduced as a pilot after which adjustments were made as needed for improved learning outcomes as well as stronger efficacy in the area of inquiry-based instruction. A year later, once a new cohort had entered the program, the adjusted materials were implemented during the quasi-experimental full study.

Because this content was new to the Early College program, this study lacked previously developed measures of learning outcomes that could be used to compare gains between this study's participants and other groups. For this reason, both the pre and posttest for content knowledge and the pre- and post-assessment for student motivation had to be developed or identified for purposes of this study. As has been described, the test for content knowledge was developed by the researcher by pulling from strongly related materials, and the assessment for student motivation was adopted during the literature review process.

Another limitation for this study was the lack of control group. Other similar studies which have experienced the same limitation addressed it by making comparisons to previous classes during which the content had been provided in a traditional manner, or they monitored increases in student scores over successive cohorts (with minimal adjustments to the pedagogy and curriculum for optimum student learning). In the case of this study, although there were two sections (morning and afternoon), since part of the purpose involved implementing this curriculum so students may earn college credit for the course, it would have been unethical to deprive one group of students from this opportunity. Second, again because the curriculum was new for the Early College program, there was not an appropriate comparison group from previous years. As the program does now incorporate this curriculum regularly, it would be possible to measure student gains over successive cohorts as was reported by Bethel and Lieberman (2014); however, that is beyond the scope of this particular study due to time constraints.

This study is also limited by minimal qualitative data to help support and flesh out the findings from the qualitative measures of content knowledge and student motivation. This is more so a function of limited time in the development, piloting, revision, and implementation of the unit as well as the collection and analysis of data. Also, the data analyses are limited by small sample sizes. This aspect of the study was taken into consideration when selecting data analysis methods.

Due to the sample being one of convenience, it is important to note potential biases. The pretest and instructional phase of the curriculum were implemented by the researcher herself who is moderately versed in the concepts and theory related to inquiry-based instruction and advanced in content knowledge, but a novice in the administration of such pedagogy. The

summative assessment and posttest phase were proctored by the Early College educator who is minimally versed in both inquiry concepts and administration, and content knowledge. These two realities likely impact the fidelity of the study, though one could argue it is a reality many educators are likely to experience should they adopt such a curriculum.

By virtue of the purpose of this study, this curriculum was implemented with students who had very minimal, if any, experience with inquiry-based learning methods. Additionally, the students involved were aware of the observations taking place. Both of these items may have resulted in some reactivity or Hawthorne effect. There was potential for some students to become frustrated by the new educational method or for some to attempt to perform better due to their awareness. Both changes in behavior may have created results which are simply due to the implementation of the intervention rather than the pedagogy itself.

Finally, the study was likely limited by the natural day to day issues of the classroom. The study was restricted by a typical classroom schedule and the researcher had no control over student attendance. There was some considerations and adjustments made for the particular needs of the students and spontaneous issues with equipment or results that were typical of science laboratory work. However, any adjustments or considerations made were ethically-driven and accounted for during data collection.

Student content knowledge. The first major hypothesis in this study was that student content knowledge would increase from pretest to posttest after the implementation of an inquiry-based biotechnology curriculum. The majority of research on inquiry-based science instruction, generally, has displayed associated improved student learning outcomes (Berns & Lawton, 2004; Duschl, Shouse, & Schwingruber, 2007; Jorgenson, 2005; Lumpe, Czerniak, & Haney, 1999; Price & Felder, 2007). Several researchers have shown inquiry-based learning

results in higher content knowledge gains when compared to traditional classrooms at educational levels ranging from elementary to post-secondary (Bredderman, 1983; Casotti, Reiser-Danner, & Knabb, 2008; Lewis & Lewis, 2008; Minner, Levy, & Century, 2010; Schnieder, Krajcik, Marx, & Soloway, 2002; Shymansky, Kyle, & Alport, 1983; Von Secker & Lissitz, 1999; Wilson, Taylor, Kowalski, & Carlson, 2010). Although the research is more limited in the area of inquiry-based biotechnology instruction, specifically, the same results were true regarding content knowledge gains (Bigler & Hanegan, 2011; Lesmes Celorrio, Fernandez Gomez-Chacon, & Gonzalez-Soltero, 2013; Movahedzadeh, Patwell, Rieker, & Gonzalez, 2012; Taraban, Box, Myers, Pollard, & Bowen, 2007).

The results of this study are consistent with previous research in that there was a significant difference between pre- and posttest ($p < .000$) and the results of this study indicate, with 95 percent confidence, that student content knowledge did increase after the implementation of an inquiry-based biotechnology curriculum by approximately 9 to 19 percentage points. It is important to keep in mind, these are content gains in an area that was previously not included in the curriculum, and they were content gains that proved strong enough to warrant awarding four semester credits worth of college credit for an introductory biotechnology course at the local four-year university. In looking at student assignment scores, the assignment average mean (including the final presentation grades) was 80.10 percent with a standard deviation of 7.94 percent, correlating with a B- average on the college course grading scale. Student comments from the survey revealed they very much enjoyed the hands-on laboratory agarose gel electrophoresis procedure, something that had not been included in the previous curriculum. Moreover, the groups obtained strong results in this area, similar to what one would expect from a university freshman course with students experiencing this for the first time. Sample gels can

be found in Figure 7 below. The mean score on the final presentation alone (of which a sample can be found in Appendix E) was 92.78 percent with a standard deviation of 6.86 percent, correlating with an A- average on the college course grading scale.

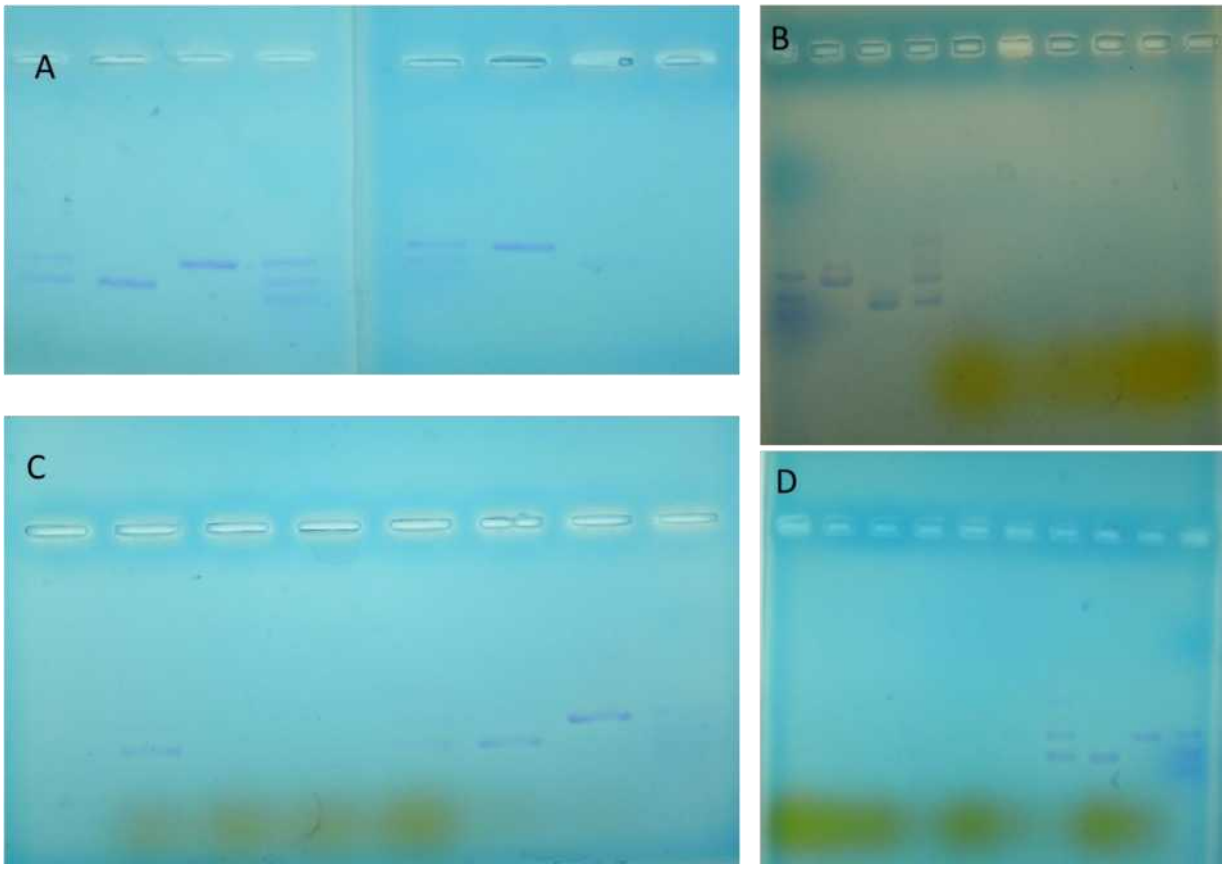


Figure 7. Sample results of agarose gel electrophoresis. Group are indicated as A) AM #1, B) AM #2, C) PM #1, and D) PM #4. Multiple band lanes indicate molecular ladder. Single higher base pair (upper) band indicates homozygous (+/+) *Alu* insert present genotype. Single lower base pair (lower) band indicates homozygous (-/-) *Alu* insert not present genotype. Double bands indicates heterozygous (+/-) genotype.

As previously described, one limitation to this study was a lack of a comparison group. Other studies with a similar challenge include those conducted by Ketpichainarong, Panijpan, and Ruenwongsa (2010), and Gunn, Seitz McCauslin, Staiger, and Pirone (2013). In both cases, the researchers implemented curricular redesigns and reported significantly ($p < 0.001$) higher posttest scores compared to the pretest. Bethel and Lieberman (2014) also structured their research in this way comparisons of pre and posttests showed marked improvements in student achievement (36 +/- 15% on pre-tests compared to 80 +/- 11% on post-tests) after several successive cohorts of students. Even in studies that did include control groups, the gains in the traditional classrooms were modest compared to those in the inquiry-based classrooms. Knight and Wood (2005) reported gains of 9 percentage points in the inquiry classroom which were found to be significantly higher ($p = .001$) than the traditional classroom. Macalalag, Brockway, McKay, and McGrath (2010) found gains of 7.6 percentage points in their inquiry classrooms, which was significantly higher than the control group who only gained by 2.7 percentage points ($p = .017$). Taken together and despite lacking a control group, the results of this research study which indicates an approximate 9 to 19 percentage point increase in content knowledge, can be considered strong and relevant for the case of inquiry-based instruction, particularly with a biotechnology curriculum.

Students' motivation toward their science learning. The second major hypothesis in this study was that student motivation toward their science learning would be more positive after participation in an inquiry-based biotechnology curriculum. Attempting to measure change in students' motivation toward their science learning using the SMTSL indicated no change when assessing overall perception. The results also indicate no change when breaking out the various components of student perception, except in the case of self-efficacy, which was defined as

students' belief in their ability to perform well in science, in which there was a significant, albeit it slight decrease from a median of 30 on the pre-assessment to a median of 28 on the post-assessment.

Although the use of inquiry-based instruction has often been found to correlate with increased interest and motivation towards science learning (Haury, 1993; Herman & Knobloch, 2004; Wilke & Straits, 2005; Witt & Ulmer, 2010), it is perhaps not surprising that we did not see measurable gains in this dimension with just one inquiry-based unit. As the units leading up to this one were largely traditional, involving introduction of vocabulary at the forefront of the lesson and the majority instruction being lecture-based, it is reasonable to expect that it would take students some time to become accustomed to this new method of learning. This is consistent with the work by Spronken-Smith, Bullard, Ray, Roberts, & Keiffer (2008) that indicates increased time commitment, group work, and a lack of security in both format and content can serve as hurdles that students must overcome when beginning inquiry-based instruction. These can all affect a student's motivation for learning science and their level of self-efficacy, or their belief in their ability to perform well, in science learning specifically.

Another contributing factor here could be prior knowledge. In terms of prior content knowledge, students came from varying backgrounds and school districts. As a result, some students already had some familiarity with biotechnology and agarose gel electrophoresis, through experience in an advanced placement biology course for example, while for others this content was all new. In the Early College course, specifically, students progressed through earlier units just as any other years' cohort had. It was common practice for this course to preface lessons with vocabulary worksheets. However, a typical vocabulary worksheet for biotechnology and agarose gel electrophoresis was not provided. Additionally, the regular instructor was also

directed by the researcher to not do anything additional in preparing these students that would be out of the norm. This all may have seemed very disconcerting to the students. This study examined just one unit of instruction, and a novel one at that in both content and pedagogy, so it very likely stood out from the pattern the students were familiar with leading up to this point. The students may have felt somewhat uncomfortable and it likely had an impact on their motivation for learning, particularly their self-efficacy or their confidence toward science learning. This is supported by student comments from the survey such as:

"I would have been much more confident about the unit if I had been given some background knowledge on the topic…"

"This unit could be better if we knew more vocabulary."

These findings are also consistent with the challenges identified by Edelson, Gordin, and Pea (1999) regarding the successful engagement of students in inquiry-based activities, one of which stated that because inquiry learning is often more challenging when compared to traditional learning activities, a higher level of motivation may be demanded of the learner. Inquiry learning also demands students maintain a level of motivation over an extended period compared to traditional instruction. To combat some of the challenges related to student motivation for learning, Edelson, Gordin, and Pea (1999) recommend educators select a meaningful problem which will have implications that matter to students as a way of establishing initial motivation. They also recommend educators design bridging activities and structured staging activities or investigations which will provide the background knowledge needed for later stages of learning. The curriculum in this study directed students through introductory exercises and videos with associated questions. It also provided scaffolding when it came to the particular technique of agarose gel electrophoresis whereby it allowed students to explore and

experiment with benign samples of food dye before having them investigate their own genotype using human DNA samples. It was clear from student comments on the survey such as:

"Hands on lab work is my favorite. It is the biggest reason I take science classes."

"I liked being given the chance to get a hands on experience from the electrophoresis lab."

that the majority of them found this all very interesting and relevant. However, the bridging and staging activities, and content relevance, may not have been enough to assuage students' discomfort with the new content and format.

Spronken-Smith, *et al*. (2008) suggest educators can mediate these hurdles by addressing them at the forefront of instruction, by providing students with the purpose and goals of inquiry-based instruction. By explaining the benefits of inquiry-based learning to students at the beginning of the lesson and providing necessary knowledge in terms of both format and content, educators may be able to ameliorate the reservations students have toward this type of learning activity and maintain student motivation over the course of the lesson. Admittedly, more explanation of inquiry-based instruction could have been provided to this group of students. This information was not necessarily intentionally reserved from the students, but it was not overtly provided in an effort to reduce threats to internal validity.

The curriculum and pedagogy for this study may have also unintentionally fallen victim to one of the warnings given regarding inquiry-based instruction which highlights the fact that many teachers still incorrectly view inquiry as simply allowing students to do hands-on activities. Thus, for successful implementation, instructors need sufficient professional development to help them better understand inquiry instruction as well as sustained support (Blanchard et al., 2009; Crawford, 2000, 2007; Luft, 2007; Windschitl, 2004). The pedagogy developed did attempt to layer levels from 'structured' to 'guided' in an effort to introduce

students to the practice of inquiry. However, this may not have been enough support to students who may have never experienced this form of learning previously. This concern is particularly evident in some of the student comments shown below that were taken from a survey informally given at the conclusion of the study.

Asking, "Putting your feelings about the instructor aside, in what ways could this unit be improved?" the following were some of the more indicative responses:

"Give out a review, and a vocabulary list."

"...going over homework the next day."

"We could have talked more about how electrophoresis and PCR work."

"More organization as far as class structure."

"The organization."

"Explaining the literal purposes of techniques and stuff we used more."

Asking, "Putting your feelings about the unit aside, in what ways could the instructor improve?" the following were some of the more indicative responses:

"Review more (periodically & before moving on)."

"Going over vocabulary [and] what to study."

"She could have given us a vocabulary list before the unit so we would feel more comfortable with the words and the content."

"Went more in depth with the labs (instructions)."

"More pre info on what is actually on the test."

"Explain instructions better."

Interestingly though, although motivation is often cited as one of its challenges, inquiry-based instruction has the ability to motivate students for their own learning, regardless of

whether the proper result occurs (Wilke & Straits, 2005; Witt & Ulmer, 2010). This occurred in this study. Some of the students were not able to obtain viable results in their runs of agarose gel electrophoresis. This was likely primarily due to low sample volume, but it did not dissuade the students. Many of them were still very interested in the results achieved by their peers and in further understanding the implications of the procedure and its results. These observations are particularly evident in some of the student comments shown below that were taken from the same survey referenced above.

Asking, "Putting your feelings about the instructor aside, what did you like best about this unit?" the following were some of the more indicative responses regarding their interest in biotechnology:

"That we used our DNA…"

"I liked the amount of independence we were given, and the gel electrophoresis lab we did."

"I am a visual learner - so it was cool to see [referencing the gel electrophoresis activity]."

"I liked how hands on it was and how we actually got to do the things we were learning about."

"The wonderful lab days!"

"The hands on part of it made it easier to understand."

Asking, "Putting your feelings about the unit aside, what did you like best about the instructor?" the following were some of the more indicative responses of their level of security in class:

"She was willing to help if needed."

"I enjoyed her ability to sit back and let us problem solve."

"She always made sure everyone was involved - no one could just not do their homework or not participate in a lab. She made us all be accountable for our work."

"She has a lot of example that can make you understand easier."

"She pushed us to learn and figure it out ourselves."

"...put the information out there and left us to our own devices to ask for help if we needed it."

"Hands on teaching."

Although in this study student motivation, as measured using the SMTSL, did not show measureable changes from pre-assessment to post-assessment, the comments from the students seemed to indicate that the curriculum was well-received. Taken together, this is interpreted to mean that although students were likely uncomfortable with the new instructional methods, they were still very much interested in both the new content and how they were approaching the material.

Overall findings. Through the course of analysis, it quickly became evident that student content knowledge and students' motivation toward science learning could be related. The decision was also made to incorporate student assignment grades as a direct measure of the curriculum itself. In exploring a model of path analysis relating these variables to each other, the following results were found: 1) 53.8 percent of the variability in students' motivation toward their science learning after the biotechnology curriculum could be explained for by a student's previous motivation toward their science learning, and 2) 36.7 percent of students' posttest scores could be explained by a student's previous content knowledge, their performance throughout the curriculum, and their final motivation for science learning. These results support previous research demonstrating the importance of motivation and other affective components of cognitive growth to student learning and skill development in the areas of science learning achievement (Napier & Riley, 1985; Tuan, Chin, & Shieh, 2005), and critical thinking and learning strategies (Garcia & Pintrich, 1992; Kuyper *et al*., 2000; Wolters 1999).

Reflecting on the results regarding content knowledge and student motivation, this study found that while significant gains were achieved in content knowledge, gains in student motivation were not achieved in a measurable way, though student survey comments demonstrated an overall positive response to the new curriculum. The results of the path analysis highlight the importance of 1) students' beginning motivation to ending motivation toward science learning, and 2) ending motivation, assignment performance, and previous content knowledge to final content knowledge. Taken together, we conclude that there is an avenue for more work to be done with this curriculum in order to address the affective components that are important to cognitive growth, and in doing so, there is potential for even larger content knowledge gains and increased student motivation toward science learning.

Implications and Recommendations

This study reports the results of implementing an inquiry-based biotechnology curriculum to enable students in an Early College program to construct their own understanding of DNA and chromosomal structure, genes, genetic traits and heredity, DNA isolation, polymerase chain reaction (PCR) amplification, agarose gel electrophoresis, human *Alu* PV92, genetic diseases and disorders, pharmacogenomics, and the analysis of scientific data and communication of scientific information. In a guided-inquiry environment, students appreciated the ability to work with what otherwise has been deemed advanced content. This inquiry-based biotechnology curriculum met the needs of both students and faculty in terms of their content knowledge gains and has made a strong contribution in promoting constructive learning, and thus has become a permanent instructional unit in the Early College program. It is expected to promote student skills and the critical thinking demanded in the pursuit of university programs in the medical, science, and biotechnology fields.

One of the primary hesitations on the part of instructors when it comes to the decisions about implementing inquiry-based instruction is the fear that many instructors view inquiry-based instruction as an approach that requires significant time and materials to develop and more time and effort on the part of the students (Moss, 1997), and a method that is difficult to manage in traditional classroom environments (Barab & Luehmann, 2003; Henry, 1996; Stake & Easley, 1978). This biotechnology unit provides an example of instruction that, while it demands about three weeks of regular classroom time, includes coverage of a number of topics that were previously not incorporated into the science curriculum. Although there was some cost to its implementation, the cost was no higher than it would be should an instructor want to cover the material in a traditional classroom environment. And, classroom management was similar to the experience of any laboratory environment where the instructor moves about the classroom in order to be present with each student group and address questions or conversations as they arise.

Other hurdles to the implementation of inquiry instruction cited by critics include issues such as a lack of aligned curriculum materials and the paucity of assessments capable of measuring new performance expectations (Penuel, Harris, & Haydel DeBarger, 2015). This biotechnology unit provides an example of instruction that has been aligned with various standards concerning both secondary and post-secondary education, and which includes both formative and summative assessments to enable the instructor to measure performance expectations. The pre and posttest used for purposes of the study itself combined questions taken from common texts often used in secondary biology and advanced biology classrooms as well as introductory biology courses found at the post-secondary level, McGraw-Hill Companies' *Human Genetics: Concepts and Applications, 9th edition* (Lewis, 2009), and Pearson Education's *Campbell Biology, 9th edition* (Reece, Taylor, Simon, & Dickey, 2010), as well as

the College Board's (1999) Advanced Placement (AP) Biology exam. While there may be debate as to how well these materials are aligned with new performance expectations, such as those found in the *Next Generation Science Standards*, it does provide a starting point.

Similarly, many critics include the concern for professional development as a hurdle for implementing inquiry-based instruction (Penuel, Harris, & Haydel DeBarger, 2015). This is an area that should be further addressed. The curriculum used in this study was implemented by a graduate research student who had become familiar with the characteristics of inquiry instruction and in how it differs from traditional pedagogy. Still, when actually having to implement it, there were times at which the researcher was feeling her way. And, we would be remiss if we did not consider this as contributing to the results concerning students' motivation toward science learning. Spronken-Smith, *et al*. (2008) suggest that to properly facilitate inquiry based learning, instructors must use particular questioning techniques that should stimulate idea generation while demonstrating a genuine interest in what student have to say. This questioning technique will demonstrate support for the students and provide them with clues as to whether they are "on track". This questioning technique would have significant implications for students' self-efficacy and, as was evidenced by some of the comments taken from the student surveys, may have made a difference in their post-assessment scores in this study. Appropriate professional development should be provided to teachers in order to assist them in developing these skills and a comfort level with this sort of questioning technique.

Hurdles identified to implementing biotechnology curriculum closely mirror those cited about implementing inquiry-based instruction. Teachers are often hesitant due to concerns in both subject matter knowledge and instructional practices and curricula (Borgerding, Sadler, & Koroly, 2013). There are considerable misconceptions regarding what biotechnology entails with

instructors often citing topics generally included in general biology courses such as DNA structure and function; genes and heredity; adaptation and speciation; Mendelian genetics; chromosomal theory; and cell biology (Borgerding, Sadler, & Koroly, 2013). Biotechnology-specific topics such as bioethics, applications within medicine, the environment, and agriculture, career opportunities, advantages and limitations of biotechnology, and the impact on humans were referenced only minimally (Brown et al, 1998; Zeller, 1994). When asked about how they deliver biotechnology instruction in their classrooms, teachers most often cited lecture or discussion methods (32.6%), followed by laboratories (21.4%), and audiovisual methods (17.5%) (Zeller, 1994).

This study provides an example of a curriculum that begins to bridge some of these gaps in terms of both subject matter and instructional methods. The content include topics from both general biology such as genes and heredity, adaptation and speciation, Mendelian genetics, and chromosomal theory, while offering and introduction to biotechnology-specific topics such as bioethics, applications within medicine, advantages and limitations of biotechnology, and the impact on humans. Especially for students of either varying prior knowledge or experience with inquiry instruction or both, this sort of scaffolding and layering is meant to introduce and build upon both content knowledge and inquiry skills. As recommended, this takes students from the familiar to unfamiliar with a minimum level of guidance in order to engage students in the process of learning and to reap the gains of inquiry instruction (Dalton et al., 1997).

Areas for Further Research

Evidence of significant content knowledge gains and student interest in performing hands-on laboratory work rather than having it demonstrated for them, as evidence by student survey responses, indicates that inquiry-based biotechnology curriculum is an area worth further study. However, this study revealed a reoccurring theme concerning the effectiveness of inquiry-based instruction in biotechnology to generate gains in students' motivation toward science learning. Although the results of the study were consistent with previous research concerning the important of student motivation for science learning, a similar study conducted where more careful attention is paid to this affective component of learning would be beneficial to educational leaders and to those individual teachers looking to implement such a curriculum in their own classrooms.

Changes in the study design would also be beneficial. A primary limitation of this study was that there was no control group. Therefore, no comparisons could be made between treatment and control - between inquiry-based biotechnology instruction and biotechnology instruction in a traditional classroom. With the current study, there was no way of knowing if the changes in content knowledge were directly due to the curriculum that was implemented. It is possible that the curriculum had an indirect impact on student learning by, for example, encouraging different study habits or by encouraging more study time or group work out of class. Future studies could do more to uncover the particular mechanisms that lead to student content knowledge gains. Again, having a control group would help with some of this, but even finer grained assessments would need to be developed to determine the particular theoretical base that is responsible for differences that might be found between treatment and control.

By scaling up the study the inquiry-based biotechnology curriculum could be further assessed for its effectiveness. Only one cohort, two classes totaling forty-six students, was used in this study. Also, the students in this study were part of a specialized population; they were students who showed both an academic affinity for as well as a personal interest in the sciences and the medical or biotechnological field. Expanding the study to include more students and expanding the study to include more student populations would be beneficial to understanding the effectiveness of such a curriculum. A study of curriculum effectiveness would also benefit from a long term study to assess both the sustainability of students' interest in science and biotechnology as well as the progression of outcome measures from year to year.

Appendix A

Institutional Review Board Exempt Approval Memo (begins next page)

DATE:	November 26, 2013
TO:	Deborah Herrington
FROM:	Grand Valley State University Human Research Review Committee
STUDY TITLE:	[536385-1] Inquiry-Based Biotechnology Education for Kent Intermediate School District Early College Program
REFERENCE #:	14-097-H
SUBMISSION TYPE:	New Project
ACTION:	EXEMPT
EFFECTIVE DATE:	November 26, 2013
REVIEW TYPE:	Exempt Review

Thank you for your submission of materials for your planned research study. It has been determined that this project: *IS COVERED* human subjects research* according to current federal regulations and MEETS eligibility for exempt determination under category 45 CFR 46.101(b)(1).

Note: You state, *"In implementing this biotechnology unit, the project also aims to evaluate the impact of inquiry based learning on student achievement in comparison to more traditional methods of teaching."* **The HRRC struggles to understand how this might be possible since the current design includes no control group (students who have undergone traditional methods of teaching). Therefore how is it possible to make any grounded comparison of the Inquiry-based instruction to traditional instruction?**

Exempt protocols do not require formal approval, renewal or closure by the HRRC. Any revision to exempt research that alters the risk/benefit ratio or affects eligibility for exempt review must be submitted to the HRRC using the *Change in Approved Protocol* form before changes are implemented.

Any research-related problem or event resulting in a fatality or hospitalization requires immediate notification to the Human Research Review Committee Chair, Dr. Paul J. Reitemeier, 616-331-3417 **AND** Human Research Protections Administrator, Mr. Jon Jellema, in the Office of the Provost, 616-331-2400. See *HRRC policy 1020, Unanticipated problems and adverse events.*

Exempt research studies are eligible for audits.

If you have any questions, please contact the Research Protections Program, Monday through Thursday, at (616) 331-3197 or rpp@gvsu.edu. The office observes all university holidays, and does not process applications during exam week or between academic terms. Please include your study title and reference number in all correspondence with our office.

*Research is a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge (45 CFR 46.102 (d)).

Appendix B

Materials Usage Authorization Form - Bio-Rad (begins next page)

BIO-RAD

Material Usage Authorization Form

Approval Process:

1) **Materials Usage Authorization Form** (this form, complete & sign); email form to:

Corporate Communications
tina_cuccia@bio-rad.com
Fax: +1 510-741-5817

2) **Bio-Rad Style Guidelines for Third Parties** (please review regarding Bio-Rad logo use, how to refer to Bio-Rad, product names, attribution, etc. Adherence to these guidelines is expected with the use of any Bio-Rad materials.

1. Today's date: 11/29/16
2. Your name (requestor): Meagan Treadway
3. Name of company (or organization) you represent: Grand Valley State University
4. Your phone number: 616-331-5000
5. Email: treadmea@gvsu.edu
6. Fax: n/a
7. Mailing address: Meagan Treadway Grand Valley State University 1 Campus Dr. Allendale, MI 49401
8. Individual at Bio-Rad with who, you are working with to obtain Bio-Rad materials: Ingrid Hermanson-Miller, PhD | Marketing Manager, Bio-Rad Laboratories| Life Science Group
9. Describe how the materials you are requesting will be used. Please also provide: 1) photocopies of the photos and/or images you request, 2) text that will accompany these images, and 3) an illustration that shows intended layout and design of these materials: The materials requested will be incorporated as part of the curriculum for a larger (3-week) inquiry-based biotechnology unit and addresses the following topics: DNA and chromosomal structure, genes, genetic traits and heredity, DNA isolation, polymerase chain reaction (PCR) amplification, agarose gel electrophoresis, human Alu PV92, genetic diseases and disorders, pharmacogenomics, and the analysis of scientific data and communication of scientific information. The Bio-Rad materials are a critical portion of the curriculum and are supplemented with additional content and lessons on both the front and back ends, as well as throughout, the overall unit. The research questions for the study are: 1. What are the effects of an inquiry-based biotechnology unit on students' content knowledge? 2. How do students' motivation toward science learning differ between pre- and post-assessment after engaging in an inquiry-based biotechnology unit? These research questions will be assessed using a pre- and post-test for content knowledge as well as a pre- and post-assessment for students' motivation toward science learning. Student assignment, as well as a final presentation, grades will be collected for each student as well. A short survey will also be given at the end simply to ask students what they liked and disliked the most about both the course and the instructor. Pre- and post-test for content knowledge and pre- and post-assessment for students' motivation toward science learning will be evaluated by paired samples t-test or Wilcoxon Signed Rank test using SPSS. Additional analyses will be performed that will include student assignment and final presentation scores in an effort to tie all components together. This will be done by performing path analysis via multiple regression modeling, offering a holistic view of how each variable interrelates to effect both content knowledge and student motivation. A sample of one lesson has been included in the following pages, so that you may see how Bio-Rad materials have been modified and incorporated along with other content.

10. Please indicate where the requested materials will be published below. Please mark all that apply:

X One-time publication/broadcast (please circle all that apply: book, magazine, educational materials, newspaper, literature, other)	X U.S. only
	___ International only
X Internet (see item 13 below)	___ U.S. & International
___ Tradeshow/conference	___ Other (describe):

11. If the materials will be printed, specify the number of copies to be distributed: 48

12. If the materials will be broadcast, please specify the number of times material will be broadcast: n/a

13. If the materials will be posted on the Internet, please specify the duration the material will be posted: If approved, the completed thesis (containing the materials described here) will be submitted to the University Libraries ScholarWorks Institutional (open-access) Repository (http://scholarworks.gvsu.edu)indefinitely (an embargo of up to 3 years is optional).

14. Specify the format needed for materials: paper copies included in curriculum unit workbooks

15. Resolution needed: approval of copyright permission

16. Date needed: December 23, 2016

17. Please describe any plans to modify the requested Bio-Rad material(s) in any way:

I am proposing the development of an inquiry-based biotechnology unit. This unit is structured with the following lesson schedule:

Week 1	
Monday	Lesson 1: Electrophoresis & Genetic Trait Exploration
Tuesday	Lesson 2: Dye Electrophoresis & Gene Exercises
Wednesday	Lesson 3: Class Discussion of Electrophoresis and Genetic Traits
Thursday	Lesson 4: Introduction to Human *Alu* PV92 & Isolation of Cheek Cell DNA
Friday	Lesson 5: PCR Amplification of DNA
Week 2	
Monday	Lesson 6: DNA Analysis by Gel Electrophoresis
Tuesday	Lesson 7: Analysis and Interpretation of Your Results
Wednesday	Lesson 8: Molecular Techniques and Genetic Diseases/Disorders
Thursday	Lesson 9: Introduction to Genetic Based Diseases/Disorders
Friday	Lesson 10: Pharmacogenomics
Week 3	
Recommendation: Give students 2-3 workdays so they have in-class time to work with their groups on the presentations.	
Thursday	Lesson 11: Genetic Disorder/Disease Presentations – Day 1
Friday	Lesson 11: Genetic Disorder/Disease Presentations – Day 2 (if needed)

Request for copyright permission includes portions of the following materials used and modified in the ways described below:

- Biotechnology Explorer, STEM Electrophoresis Kit, 166-5080EDU
  - Student Manual
    - Lesson 1
      - Images used in an "identifying the components" worksheet
    - Lesson 2
      - Materials and equipment list (modified)
      - Structure of reference dyes (images)
      - Protocol along with images
    - Lesson 3
      - Focus questions (modified)
  - Instructor Manual
    - Duplicate of the areas listed for the Student Manual with, additionally, modified advanced preparation instructions.
- Biotechnology Explorer, Chromosome 16: PV92 PCR Informatics Kit, 166-2100EDU
  - Student Manual
    - Lesson 3
      - "splicing of introns from genes" image along with modified text from Genes and DNA

portion used in "Introduction to electrophoresis" worksheet
- Lesson 4
  - "location of an Alu insertion within an intron" and "the presence or absence of the Alu insert within the PV92 locus on chromosome 16" images, along with modified text from Genes and DNA portion used in "What is Alu PV92" instructions
  - Materials and equipment list (modified)
  - Images along with modified protocol used in "isolation of cheek cell DNA procedure"
- Lesson 5
  - Materials and equipment list (modified)
  - Images along with modified protocol used in "PCR procedure"
  - "A complete cycle of PCR" and "schematic of PCR amplification of DNA fragments" images, along with modified text from PCR Step by Step used in "Understanding PCR" worksheet
- Lesson 6
  - Materials and equipment list (modified)
  - Images along with modified protocol used in "DNA Electrophoresis Procedure"
  - Focus questions from "Gel Electrophoresis of Amplified PCR Samples" used in "Discussion Questions" worksheet
- Lesson 7
  - "Electrophoretic separation of DNA bands based on size" image, along with modified text from "Analysis and Interpretation of Results" used in "Interpreting Your Gel"

o Instructor Manual
- Duplicate of the areas listed for the Student Manual with, additionally, modified advanced preparation instructions.

AUTHORIZATION:

Tina C... 12/15/16

Bio-Rad Corporate Communications Representative — Date — Bio-Rad Legal (if necessary) — Date

Ingrid Miller LSG SDU Marketing Manager 11/30/16

Requestor's Contact at Bio-Rad — Title /Division — Date

REQUESTOR'S SIGNATURE:

I understand that permission to use the material identified above is granted based on the information provided above. I will use the material(s) only pursuant to the information above and any conditions placed upon me by Bio-Rad Laboratories.

Signature of individual requesting material(s) — 11/29/16 Date

Lesson 1 Electrophoresis & Genetic Trait Exploration

Advanced Preparation

Prepare molten agarose (15 min)

Materials Needed for Advanced Preparation	Quantity
Electrophoresis buffer, 50x TAE	25 ml
Molecular biology grade agarose	5 g
Marking pen	1
Balance	1
Erlenmeyer flask	1
Microwave oven or hot plate	1

The recommended agarose concentration for gels in this application is 1% agarose. This concentration of agarose provides good resolution and minimizes run time required for electrophoretic separation of the dyes. The volume required for each gel is 50 ml. This volume of agarose ensures that the wells are deep enough to easily pipet 10 µl of sample into each well and that there is space above the gel to overlay with running buffer. **Be sure to use 1x TAE electrophoresis buffer, not water, to prepare agarose gels.**

1x TAE electrophoresis buffer preparation

TAE (Tris-acetate-EDTA) electrophoresis buffer is provided as a 50x concentrated solution. In addition to the 1x TAE buffer needed to prepare the agarose gels, 55 ml of buffer is also required for each electrophoresis chamber. One liter of 1x TAE buffer will be sufficient to prepare and run 8 agarose gels. To make 1 L of 1x TAE buffer from 50x TAE concentrate, add 20 ml of 50x concentrate to 980 ml of distilled water and mix. Aliquot 50 ml of buffer into smaller Erlenmeyer flasks for student use.

Agarose preparation

These procedures may be carried out 1 to 2 days ahead of time by the teacher. The instructions below are for the preparation of the molten agarose assuming the students will prepare their own gel boxes and pour their own gels.

I) To make 1% agarose solution, use 1 g of agarose for 100 ml of 1x TAE electrophoresis buffer. Be sure to use electrophoresis buffer, not water.

Use the following guide for gel volume requirements when casting single or multiple gels.

Number of gels	Volume of 1% agarose
1	50 ml
2	100 ml
4	200 ml
8	400 ml

ii) Add the agarose powder to a suitable container (for example, use a 500 ml Erlenmeyer flask for 200 ml or less). Add the appropriate amount of 1x TAE electrophoresis buffer. If clumps of agarose are visible, swirl the flask to mix.

iii) While heating the solution, some of the volume will be lost to evaporation. Prior to heating, mark the volume level on the flask or weigh the flask and content and record the weight.

iv) Dissolve the agarose by heating in a microwave oven. If a microwave is not available, the solution can be heated on a magnetic hot plate.

Caution: Always wear protective gloves, goggles, and lab coat while preparing and casting gels. Molten agarose or the flasks containing hot agarose can cause severe burns if allowed to contact skin.

a. Microwave oven method

This technique is the fastest and safest way to dissolve agarose. Place the gel solution in an appropriate bottle or flask into the microwave. If you are using a bottle, **be sure to loosen the cap before heating.** The ideal microwave setting will depend on the volume of agarose solution that you are preparing and on the power of the microwave oven. For small volumes, microwave solution for 1 min, swirl the solution, then microwave in 20-30 sec intervals, swirling after each, until all of the small transparent agarose particles are dissolved. For larger volumes (e.g. 400 ml), heat initially for 2-3 min, swirl the solution, then microwave in 30-45 sec intervals, swirling after each, until all of the small transparent agarose particles are dissolved.

Add water to the agarose solution to bring it back to the original volume and swirl to mix completely. Cool agarose solution to 55-60 °C before pouring gels. If students are going to pour their own gels, keep the agarose at 55-60 °C until it is ready to be poured by either stirring on a hot plate or in a water bath. If the agarose is kept warm in a water bath, make sure to swirl it thoroughly to mix the solution immediately before the gels are poured.

b. Magnetic hot plate method

Add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Boil the solution until all of the small transparent agarose particles are dissolved.

Add water to the agarose solution to bring it to the original volume and swirl to mix completely. Cool agarose gels to 55-60 °C before pouring gels. If students are going to pour their own gels, keep the agarose at 55-60 °C until it is ready to be poured by stirring on a hotplate. Aliquot 50 ml of molten agarose into smaller Erlenmeyer flasks for student use.

Aliquot dye extraction solution and reference dyes (45 min)

Aliquot dye extraction solution

Materials Needed for Advance Preparation	Quantity
Dye extraction solution	25 ml
2 ml Microcentrifuge tubes	8
Marking pen	1
100-1,000 µl adjustable-volume micropipette and tips or DPTPs	1
Candies	

Label eight clean 2 ml Microcentrifuge tubes **Extraction** and aliquot 2 ml of dye extraction solution into each tube.

Aliquot reference dyes

Materials Needed for Advance Preparation	Quantity
Blue 1 reference dye	150 µl
Yellow 5 reference dye	150 µl
Yellow 6 reference dye	150 µl
Red 40 reference dye	150 µl
2 ml Microcentrifuge tubes	32
2-20 µl adjustable-volume micropipette and tips	1
Marking pen	1

1. If you have a centrifuge, pulse spin the reference dyes to pool the solutions at the bottom of the tubes.
2. Allquot Blue 1 reference dye

   Label eight clean 2 ml Microcentrifuge tubes **Blue 1** and aliquot 15 µl of Blue 1 reference dye into each tube.
3. Allquot Yellow 5 reference dye

   Label eight clean 2 ml Microcentrifuge tubes **Yellow 5** and aliquot 15 µl of Yellow 5 reference dye into each tube.
4. Aliquot Yellow 6 reference dye

   Label eight clean 2 ml Microcentrifuge tubes **Yellow 6** and aliquot 15 µl of Yellow 6 reference dye into each tube.
5. Aliquot Red 40 reference dye

   Label eight clean 2 ml Microcentrifuge tubes **Red 40** and aliquot 15 µl of Red 40 reference dye into each tube.

Set up workstations (30 min)

The kit provides enough materials for 8 student workstations. Workstations should include no more than 4 or 5 students. Carefully ensure that each workstation is equipped with the following materials.

Student Workstation	Quantity
Plastic chamber	1
8-well comb	1
Ruler	1
Molten agarose	50 ml
Marking pen	1
Paper clips	2
Black lead with alligator clips	1
Red lead with alligator clips	1
9 volt batteries	3-5
Blue 1 reference dye	15 µl
Yellow 5 reference dye	15 µl
Yellow 6 reference dye	15 µl
Red 40 reference dye	15 µl
1x TAE buffer	22 ml
Dyes extracted from candies	4 samples
2-20 ml adjustable-volume micropipette and tips	1

Lesson 1 Electrophoresis & Genetic Trait Exploration

Instruction

The students should come into class where their workstations are already set up and "Identifying the Components" and "Determining Your Procedure" has been printed out for them, and are both waiting at their seat.

10 minutes Instructions

Explain that students will spend time today freely exploring agarose gel electrophoresis. They should be sure they understand the different components (Identifying the Components) needed to run such a procedure. Once they have explored the various individual components, encourage them to try setting one up and running it. While they are exploring how to run a gel, they should come up with the steps needed to run a gel in their own words (Determining the Procedure).

30 minutes Activity

The instructor should move about the classroom, interacting with students to be sure learning takes place throughout the class period. Offer assistance or ask students questions regarding the task to keep them motivated. Explore concepts such as how the agarose gel works to separate DNA, what the buffer does for the process, and why students should be aware of where the (+) and (-) electrodes are located. Allow them, as much as possible, to come to their own conclusions. Use a Socratic method of instruction to help them defend their ideas for the procedure.

20 minutes Wrap-Up

Ask the students to turn in their worksheets ("Identifying the Components" and "Determining Your Procedure") before they leave. The instructor can look these over and use them to identify points of discussion during the next day's lesson.

Introduce the homework; "Human Genetic Traits Inventory" and "Human Karyotype Exercise". Review terminology for both worksheets (allele, gene, dominant, recessive, genotype, and phenotype). Work through the first item on the Human Genetic Traits Inventory (tongue rolling) as a class so the students have an example to work from. Explain the nomenclature (RR, Rr, and rr). Remind them that if they know they are a dominant phenotype, but are unsure of their genotype, they may answer R_. Review concepts related to the Human Karyotype Exercise such as those described on the first page (chromosome length, centromere position, banding pattern, and satellite endings). These two worksheets will be due by the day of the third lesson.

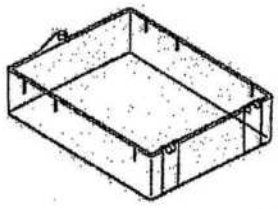
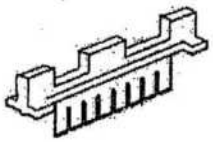
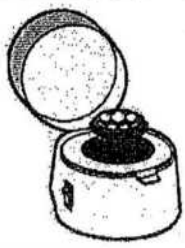
Lesson 1 Electrophoresis & Genetic Trait Exploration

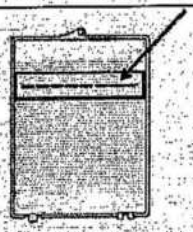
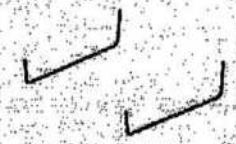
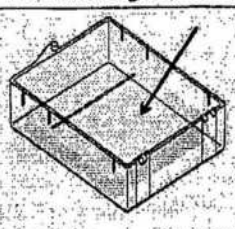
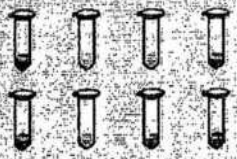
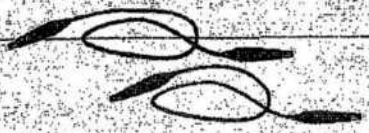
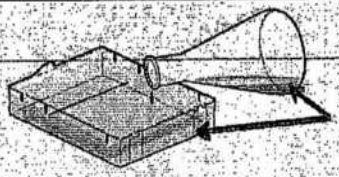
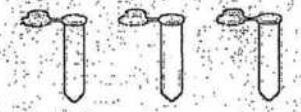
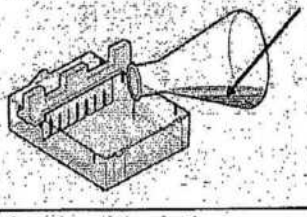
Identifying the Components

Agarose gel electrophoresis is a method that uses electrical current to separate macromolecules, such as DNA, RNA, and proteins, and their fragments based on their size and charge. Before you can use this technique, though, you need to know what materials you'll be working with during the lab. Take a look at the components, both materials and equipment, in front of you on your lab bench and at the shared table for the lab. Is there anything you recognize right away? What are some things you have never seen before? Can you work with your lab mates to identify the different components and take a guess at what they might be used for during agarose gel electrophoresis? Below are some helpful pictures and names for the different components you might encounter. See if you can match any of them with one another.

Word Bank		
Wells	Solidified Agarose Gel	Leads
Buffer	Pipet Tip	Comb
Microcentrifuge Tubes	Molten Agarose Gel	Centrifuge
Electrodes	Samples (Known and Unknown)	Pipet
Power Supply		Gel Electrophoresis Chamber

Select words from the word bank above and fill in spaces A-N to identify the pictures below them.

A: Gel Electrophoresis Chamber	B: Comb	C: Centrifuge
		

D: Wells	E: Electrodes	F: Solidified Agarose Gel
		
G: Samples (Known and Unknown)	**H: Pipet** **I: Pipet Tip**	**J: Power Supply**
		
K: Leads	**L: Buffer**	
		
M: Microcentrifuge Tubes	**N: Molten Agarose Gel**	
		

Lesson 1 Electrophoresis & Genetic Trait Exploration

Determining Your Procedure

Now that you are familiar with the different components with which you will be working, how should you move forward with your agarose gel electrophoresis procedure? What are some things you should consider related to your sample, putting together the electrophoresis apparatus, and making and preparing your gel for samples? How long do you think the gel should be run and how will you know when it is done? What might you need to do with the gel once the samples are done running? Using the space below and on the back of this page, work with your lab mates to number and describe several steps, in logical order, you would use to complete this procedure.

Suggested procedure (do not share with students):

1. Measure and mark a distance of 1 cm, on the outside of the box, from both ends of the box on both the longest sides with a marker.
2. Measure and mark a distance of 3 cm on both the longest sides from one end of the box with a marker.
3. Place the comb on the 3 cm mark and be sure it is centered in the box so none of the combs are touching the plastic box.
4. Prepare 50 ml of molten agarose and gently pour it into the box. Wait 10-20 minutes for the gel to solidify.
5. Carefully remove the comb from the solidified gel.
6. Cut the gel at each of the 1 cm marks and remove the ends of the gel from the box.
7. Prepare and add 55 ml of 1x TAE buffer to the box.
8. Construct the electrodes by bending the paperclips until they make a "U" shape and insert one at each end of the box with the arms sticking straight up out of the buffer.
9. Prepare the extracted dye samples, and using a separate tip for each color, load 10 µl of each sample, one into each of the 8 wells.
10. Assemble the battery tower by connecting the negative nodes to positive nodes.
11. Attach the black alligator clip to the long end of the paper clip at the end of the box closest to the wells, and attach the red alligator clip to the long end of the paper clip at the other end of the box.

12. Attach the other end of the alligator clips to the positive and negative terminals, respectively, on the battery tower.

13. Allow the gel to run about 20 minutes, but disconnect the alligator clips from the battery tower before the dyes run off the end of the gel.

14. Take a picture of the gel to record the results.

Notes:

Students will not normally know the volume needed for each of the reagents, which is fine at this point. They may also not understand the need to cut the gel or the role the paper clips play. Guide their understanding toward the idea that the buffer is the conductor for the electrical current from the batteries, and it is this current which allows the samples to move down the gel. The gel must be cut so that the buffer can surround the gel adequately. The paperclips act as the electrodes to help transmit this current. Ask them, "What causes the samples to move through the gel?" Ask, "What role do you think the batteries play in this procedure?" Introduce them to the idea of how samples separate by size on the gel by asking, "Why do some samples move further than others?" or "How is it that the larger sized samples will stay closer to the wells while the smaller samples will be found toward the end of the gel?" Students typically understand the need for and purpose of the comb well. Engage students in conversation regarding contamination and the importance of using separate tips for each sample. Also, ask them questions about how to know when to stop their gels. This will be an opportunity to introduce the ideas of a loading dye. The preparation of each of the reagents will also be discussed in more detail at later points in this exercise. There will also be an opportunity to review proper pipetting skills later as well.

Safety:

For the most part, there are no sever hazards with this laboratory procedure. Students should be reminded to follow standard laboratory safety procedures (do not smell or taste anything at the lab bench and wear gloves, goggles, and a lab coat if available). The most critical part to be aware of is the temperature of the molten agarose. Students to take caution in preparing and pouring the molten agarose because it will be hot and could cause severe burns if allowed to contact skin.

Otherwise, this is the opportunity for students to make mistakes and learn from them.

Lesson 1 Electrophoresis & Genetic Trait Exploration

Human Genetic Traits Example Answers

After reviewing the phenotypes on the previous page, complete the inventory below by determining your own phenotype and genotype as best you can. You may need to think about the traits in your parents to help determine the details. We will then take a look at the total inventory for our class when everyone is complete.

Trait	Symbol	Dominant Phenotype	Your Phenotype	Your Genotype	Number of Phenotypes in Class*	
					Dominant	Recessive
1. Tongue Rolling	R, r	Rolled	R_	Rolled	5	5
2. Facial Dimples	D, d	Present	dd	Not Present	7	3
3. Bent Little Finger	B, b	Bent	bb	Not Bent	8	2
4. Free Ear Lobes	F, f	Free	F_	Free	4	6
5. Mid-digital Hair	H, h	Present	H_	Present	1	9
6. Hand Clasping	L, l	Top Left Thumb	L_	Top Left Thumb	3	7
7. Widow's Peak	W, w	Present	ww	Not Present	2	8
8. Hitchhiker's Thumb	T, t	Absent	T_	Absent	4	6
9. Cleft Chin	C, c	Present	cc	Not Present	9	1
10. Eye Color	M, m	Melanin (Brown)	M_	Melanin (Brown)	5	5

** This is sample data. Actual class will yield different results.

Lesson 1 Electrophoresis & Genetic Trait Exploration

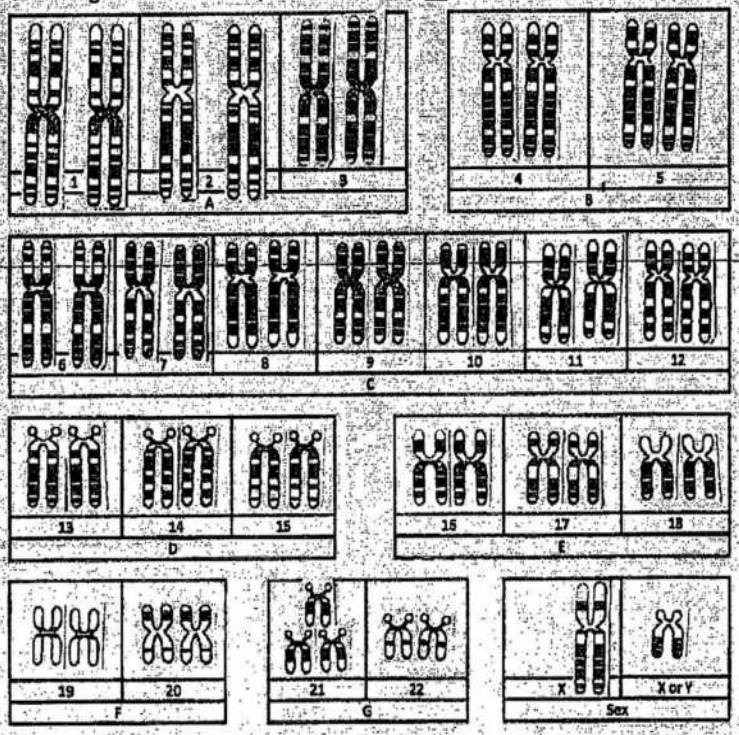
Human Karyotype Exercise Answers

For this exercise, you will be working with a partner to create your own karyotype. From the chromosome spread on the next sheet, carefully cut out each chromosome. Find the homologous pairs by matching length, the position of the centromere, and the banding pattern. Arrange the pairs on the karyotype form below in order from longest to shortest, placing the sex chromosomes at the end. Lightly tape the chromosomes in place. Once you are confident that you have them placed correctly, tape them down more securely. Then answer the following questions:

a) What is the sex of this individual: **Male** or Female ? (circle one)

b) Determine the state of the chromosomal arrangement: Normal or **Abnormal ?** (circle one)

c) If the arrangement is abnormal, state in what way: Trisomy 21 (Down Syndrome)



Appendix C

Biotechnology Unit Pre- and Post-Assessment with Answer key (begins next page)

Name: _____

Biotechnology Unit Pre- & Post-Assessment

1. ____ are the unit of heredity.

   (A) Cells
   (B) Genes
   (C) Chromosomes
   (D) Pedigrees

2. Which of the following is the genetic material of cells?

   (A) DNA
   (B) RNA
   (C) Protein
   (D) It has not been identified

3. Linda and Ben request prenatal genetic testing to determine if their unborn child has Down syndrome. Cells are collected from the fetus and the chromosomes are examined in a _____ analysis.

   (A) Pedigree
   (B) Karyotype
   (C) Multifactorial
   (D) Mendelian

4. The _____ refers to the genetic instructions (alleles present), while the _____ is the visible trait (alleles expressed).

   (A) phenotype, karyotype
   (B) karyotype, phenotype
   (C) genotype, autosome
   (D) genotype, phenotype

5. A(n) _____ allele results in an expressed trait when present in just one copy, while a _____ allele is expressed only when present in two copies.

   (A) autosomal, sex
   (B) somatic, germline
   (C) polygenic, multifactorial
   (D) dominant, recessive

6. Variations of genes are called _____, and these arise by a process called _____.

(A) alleles, mutation
(B) cells, mutation
(C) recessives, differentiation
(D) chromosomes, mitosis

7. Single nucleotide polymorphisms (SNPs) _____.

(A) are single base-pair variations in the genomes of the human population
(B) are genetics markers used to study the genetic basis for disease
(C) are small nucleotide differences among individuals located in coding and non-coding sequences in the genome
(D) can be the molecular basis for different alleles
(E) All of the listed responses are correct.

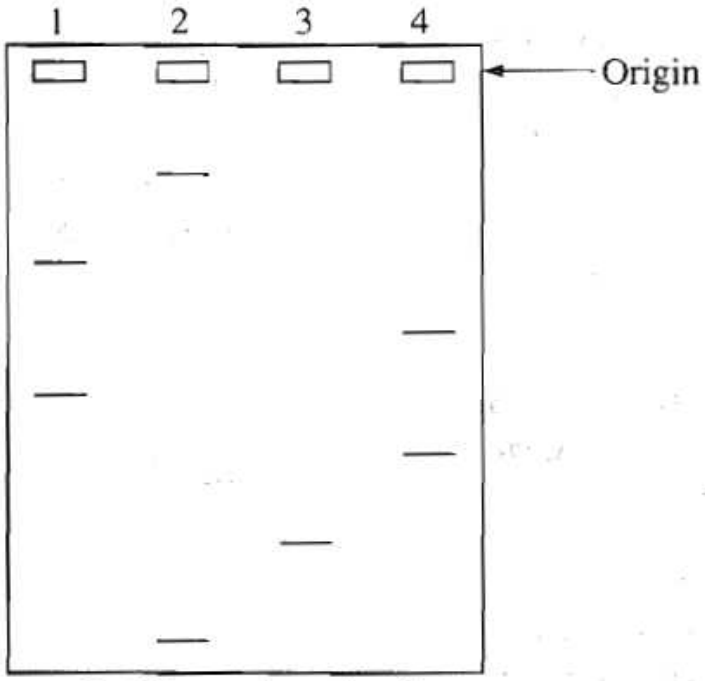
8. PCR (polymerase chain reaction) is used to amplify isolated DNA because _____.

(A) naturally occurring DNA molecules are very long and contain many genes
(B) it provides a means to produce large quantities of its protein product
(C) genes occupy only a small proportion of the chromosomal DNA in eukaryotes, the rest being noncoding nucleotide sequences
(D) it provides a means to produce many copies of a gene in a short period of time
(E) All of the listed responses are correct.

9. In the polymerase chain reaction (PCR), the sequence of bases in the primers is important because it _____.

(A) determines which segment of the genome will be amplified
(B) always matches a stop codon
(C) always causes a silent mutation
(D) determines how many cycles of the reaction are needed to obtain a sufficient amount of amplified DNA
(E) determines the number of tandem repeats in a genome

For questions 10–11, refer to an experiment that was performed to separate DNA fragments from four samples radioactively labeled with ^{32}P. The fragments were separated by gel electrophoresis. The visualized bands are illustrated in the figure below.



Gel Electrophoresis of DNA Fragments

10. The electrophoretic separation of the pieces of DNA in each of the four samples was achieved because of differential migration of the DNA fragments in an electric field. This differential migration was caused by the

(A) relative amounts of radioactivity in the DNA
(B) number of cleavage points per fragment
(c) size of each fragment
(D) overall positive charge of each fragment
(E) solubility of each fragment

11. The DNA was labeled with ^{32}P in order to

(A) stimulate DNA replication
(B) inhibit the uptake of unlabeled ATP
(c) show which fragments included the 5’ end and which fragments included the 3’ end
(D) visualize the fragments
(E) speed up the rate of separation by electrophoresis

12. Which of the following is an additional use of the gel electrophoresis technique?

(A) To express a gene
(B) To separate proteins in a mixture
(C) To ligate DNA fragments
(D) To transform E. coli
(E) To amplify genes

13. Which of the following is not a potential use for results of genetic testing?

(A) To detect genes that affect addictive behavior
(B) To diagnose and treat cancer
(C) To indicate which types of infections a patient is most susceptible to, and which antibiotics will most effectively treat them
(D) All of the above are potential uses of genetic testing

14. The technique of _____ attempts to correct certain genetic disorders.

(A) karyotype analysis
(B) risk assessment
(C) genomics
(D) gene therapy

15. DNA profiling can be used to _____.

(A) identify remains of individuals
(B) overturn convictions of innocent people
(C) determine family relationships
(D) All of the above

16. All of the following are reasons to use TRIS during an electrophoresis except:

(A) to maintain the shape of molecules.
(B) to stain molecules.
(C) to stabilize the pH.
(D) to conduct electricity.

17. Which agarose concentration would work best for separating very large molecules?

(A) 3%
(B) 0.8%
(C) 2%
(D) All choices would work well.

This questionnaire contains statements about your willingness in participating in this science class. You will be asked to express your agreement on each statement. There are no “right” or “wrong” answers. Your opinion is what is wanted. Think about how well each statement describes your willingness in participating in this class.

A. Self-efficacy	**Strongly disagree**	**Disagree**	**No opinion**	**Agree**	**Strongly agree**
1. Whether the science content is difficult or easy, I am sure that I can understand it.	1	2	3	4	5
2. I am not confident about understanding difficult science concepts. (-)	1	2	3	4	5
3. I am sure that I can do well on science tests.	1	2	3	4	5
4. No matter how much effort I put in, I cannot learn science. (-)	1	2	3	4	5
5. When science activities are too difficult, I give up or only do the easy parts. (-)	1	2	3	4	5
6. During science activities, I prefer to ask other people for the answer rather than think for myself. (-)	1	2	3	4	5
7. When I find the science content difficult, I do not try to learn it. (-)	1	2	3	4	5
B. Active learning strategies	**Strongly disagree**	**Disagree**	**No opinion**	**Agree**	**Strongly agree**
8. When learning new science concepts, I attempt to understand them.	1	2	3	4	5
9. When learning new science concepts, I connect them to my previous experiences.	1	2	3	4	5
10. When I do not understand a science concept, I find relevant resources that will help me.	1	2	3	4	5
11. When I do not understand a science concept, I would discuss with the teacher or other students to clarify my understanding.	1	2	3	4	5
12. During the learning process, I attempt to make connections between the concepts that I learn.	1	2	3	4	5
13. When I make a mistake, I try to find out why.	1	2	3	4	5
14. When I meet science concepts that I do not understand, I still try to learn them.	1	2	3	4	5
15. When new science concepts that I have learned conflict with my previous understanding, I try to understand why.	1	2	3	4	5

C. Science Learning Value	Strongly disagree	Disagree	No opinion	Agree	Strongly agree
16. I think that learning science is important because I can use it in my daily life.	1	2	3	4	5
17. I think that learning science is important because it stimulates my thinking.	1	2	3	4	5
18. In science, I think that it is important to learn to solve problems.	1	2	3	4	5
19. In science, I think it is important to participate in inquiry activities.	1	2	3	4	5
20. It is important to have the opportunity to satisfy my own curiosity when learning science.	1	2	3	4	5
D. Performance Goal	**Strongly disagree**	**Disagree**	**No opinion**	**Agree**	**Strongly agree**
21. I participate in science courses to get a good grade. (-)	1	2	3	4	5
22. I participate in science courses to perform better than other students. (-)	1	2	3	4	5
23. I participate in science courses so that other students think that I'm smart. (-)	1	2	3	4	5
24. I participate in science courses so that the teacher pays attention to me. (-)	1	2	3	4	5
E. Achievement Goal	**Strongly disagree**	**Disagree**	**No opinion**	**Agree**	**Strongly agree**
25. During a science course, I feel most fulfilled when I attain a good score in a test.	1	2	3	4	5
26. I feel most fulfilled when I feel confident about the content in a science course.	1	2	3	4	5
27. During a science course, I feel most fulfilled when I am able to solve a difficult problem.	1	2	3	4	5
28. During a science course, I feel most fulfilled when the teacher accepts my ideas.	1	2	3	4	5
29. During a science course, I feel most fulfilled when other students accept my ideas.	1	2	3	4	5

F. learning Environment Stimulation	**Strongly disagree**	**Disagree**	**No opinion**	**Agree**	**Strongly agree**
30. I am willing to participate in this science course because the content is exciting and changeable.	1	2	3	4	5
31. I am willing to participate in this science course because the teacher uses a variety of teaching methods.	1	2	3	4	5
32. I am willing to participate in this science course because the teacher does not put a lot of pressure on me.	1	2	3	4	5
33. I am willing to participate in this science course because the teacher pays attention to me.	1	2	3	4	5
34. I am willing to participate in this science course because it is challenging.	1	2	3	4	5
35. I am willing to participate in this science course because the students are involved in discussions.	1	2	3	4	5

Appendix D

Biotechnology Unit Plan Instructor's Manual (begins next page)

Grand Valley State University and Kent Intermediate School District Early College Program's

Biotechnology Unit Plan

Instructor's Manual

Developed by Meagan Treadway

In Collaboration with

Dr. Deborah Herrington - GVSU

Russell Wallsteadt - KISD

Table of Contents

Student's Manual

Introduction

Learning Objectives

Unit Objectives

- Define the terminology related to molecular biology and use these concepts when discussing how molecular techniques may be used to understand genetic disorders or diseases.
- Identify ways in which biotechnology is applied to various fields of research.
- Perform agarose gel electrophoresis technique with minimal supervision and evaluate and interpret the results.

Next Generation Science Standards

High School (9-12) Life Sciences

HS-LS3 Heredity: Inheritance and Variation of Traits

1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
2. Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.
3. Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

HS-LS4 Biological Evolution: Unity and Diversity

1. Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.
4. Construct an explanation based on evidence for how natural selection leads to adaptation of populations.
5. Evaluate the evidence supporting claims that changes in environmental conditions may result in: (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species.

National Healthcare Foundation Standards and Accountability Criteria

Foundation Standard 1: Academic Foundation

Healthcare professionals will know the academic subject matter required for proficiency within their area. They will use this knowledge as needed in their role. The following accountability criteria are considered essential for students in a health science program of study.

1.1 Human Structure and Function

1.13 Analyze the interdependence of the basic structures and functions of the human body as they relate to wellness, disease, disorders, therapies, and care/rehabilitation.

1.2 Diseases and Disorders

1.22 Research common diseases and disorders of each body system (prevention, pathology, diagnosis, and treatment).

1.23 Research biomedical therapies as they relate to the prevention, pathology, and treatment of disease.

1.3 Medical Mathematics

1.32 Analyze diagrams, charts, graphs, and tables to interpret healthcare results

Foundation Standard 2: Communications

Healthcare professionals will know the various methods of giving and obtaining information. They will communicate effectively, both orally and in writing.

2.1 Concepts of Effective Communication

2.13 Report subjective and objective information.

Foundation Standard 3: Systems

Healthcare professionals will understand how their role fits into their department, their organization and the overall healthcare environment. They will identify how key systems affect services they perform and quality of care.

3.1 Healthcare Delivery Systems

3.13 Assess the impact of emerging issues on healthcare delivery systems.

Foundation Standard 4: Employability Skills

Healthcare professionals will understand how employability skills enhance their employment opportunities and job satisfaction. They will demonstrate key employability skills and will maintain and upgrade skills, as needed.

4.2 Key Employability Skills

4.21 Apply employability skills in healthcare.

Foundation Standard 6: Ethics

Healthcare professionals will understand accepted ethical practices with respect to cultural, social, and ethnic differences within the healthcare environment. They will perform quality healthcare delivery.

6.1 Ethical Practice

6.12 Recognize ethical issues and their implications related to healthcare.

Michigan Career & Technical Education Standards (26.0102 Biotechnology Medical Sciences)

Technical Standards

I. Academic Foundation

A. Human Structure and Function

3. Analyze the interdependence of the basic structures and functions of the human body as they relate to wellness, disease, disorders, therapies, and care/rehabilitation

B. Diseases and Disorders

1. Compare diseases/disorders including respective classification(s), prevention, causes, pathogenesis, diagnoses, therapies, and care/rehabilitation

2. Investigate biomedical therapies as they relate to the prevention, pathology, and treatment of disease

3. Discuss complementary/alternative health practices as they relate to the prevention and treatment of disease

C. Medical Mathematics

1. Apply mathematical computations related to healthcare procedures

5. Analyze diagrams, charts, graphs, and tables to interpret healthcare results

II. Communications

A. Concepts of Effective Communication

5. Report relevant information in order of occurrence

8. Report subjective and objective information

9. Use medical terminology to communicate information including data and observations

C. Written Communication Skills

2. Organize technical information and summaries

III. Systems

A. Healthcare Delivery Systems

5. Explain the impact of 21st century emerging issues such as technology, epidemiology, bioethics, and socioeconomics on healthcare systems

IV. Employability Skills

B. Key Employability Skills

2. Exemplify professional characteristics

D. Employability Preparation

2. Execute work assignments and formulate solutions to problems using critical thinking skills

VI. Ethics

A. Legal and Ethical Boundaries

4. Recognize ethical issues and their implications related to healthcare

VII. Safety Practices

B. Personal Safety

2. Apply proper use of personal protective equipment (PPE)

C. Environmental Safety

3. Understand proper safety techniques to prevent accidents and to maintain a safe work environment

XI. Information Technology Applications

A. Health Information Management

4. Understand the content and diverse uses of health information

Pathway Standards

II. Academic Foundations

A. Biotechnology R&D professional will be knowledgeable in the fundamentals of biochemistry, cell biology, genetics, mathematical concepts, microbiology, molecular biology, organic chemistry, and statistics

1. Apply Mathematical concepts

a. Illustrate the concepts of percentages and ratios using a biotechnology application

3. Understand Genetics

b. Construct a karyotype with human chromosomes

c. Differentiate the genetic inheritance of a dominant homozygous trait (e.g. dwarfism) from a heterozygous (e.g. sickle cell anemia)

5. Apply principles of biochemistry

c. Describe the relationship between biochemistry and biotechnology product development

7. Apply principles of molecular biology

c. Describe the central dogma of molecular biology and how understanding this process impacts biotechnology research and development

Career & Employability Standards

I. Applied Academic Skills

A. Reading, English & Language Arts

2. Give a verbal report on reading from a technical manual

III. Developing and Presenting Information

A. Developing and Presenting information

1. Gather, interpret, analyze, and refine data

2. Analyze and synthesize information and data from multiple sources

6. Practice and demonstrate presentation skills using a variety of media and interpretive data

V. Personal Management

A. Responsibility

1. Demonstrate regular attendance, promptness, and staying with a task until satisfactory completion

2. Complete assignments with minimum supervision and meet deadlines

3. Use mistakes as learning opportunities; demonstrate persistence and adaptability to change

VI. Organizing Skills

A. Time

1. Determine goals and develop an action plan to accomplish them within a given time frame

XI. Technology Skills

B. Social, Ethical, and Human Issues

8. Adhere to fair use and copyright guidelines

9. Create appropriate citations for resources when presenting research findings

E. Technology Research Tools

3. Determine if online sources are authoritative, valid, reliable, relevant, and comprehensive

4. Distinguish between fact, opinion, point of view, and inference

Previous Knowledge Required

Next Generation Science Standards

Middle School (6-8) Life Sciences

MS-LS1 From Molecules to Organisms: Structures and Processes

1. Conduct an investigation to provide evidence that living things are made of cells; either one cell or many different numbers and types of cells.

2. Develop and use a model to describe the function of a cell as a whole and ways parts of cells contribute to the function.

5. Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms.

MS-LS3 Heredity: Inheritance and Variation of Traits

1. Develop and use a model to describe why structural changes to genes (mutations) located on chromosomes may affect proteins and may result in harmful, beneficial, or neutral effects to the structure and function of the organism.

MS-LS4 Biological Evolution: Unity and Diversity

4. Construct an explanation based on evidence that describes how genetic variations of traits in a population increase some individuals' probability of surviving and reproducing in a specific environment.

High School (9-12) Life Sciences

HS-LS1 From Molecules to Organisms: Structures and Processes

1. Construct and explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

4. Use a model to illustrate the role of cellular division (mitosis) and differentiation in producing and maintaining complex organisms.

National Healthcare Foundation Standards and Accountability Criteria

Foundation Standard 1: Academic Foundation

Healthcare professionals will know the academic subject matter required for proficiency within their area. They will use this knowledge as needed in their role. The following accountability criteria are considered essential for students in a health science program of study.

1.1 Human Structure and Function

1.11 Classify the basic structural and functional organization of the human body including chemical, cellular, tissue, organ, and system.

1.3 Medical Mathematics

1.31 Apply mathematical computations related to healthcare procedures (metric and household, conversions and measurements).

Foundation Standard 7: Safety Practices

Healthcare professionals will understand the existing and potential hazards to clients, co-workers, and self. They will prevent injury or illness through safe work practices and follow health and safety policies and procedures.

7.4 Common Safety Hazards

7.42 Comply with safety signs, symbols, and labels

Foundation Standard 8: Teamwork

Healthcare professionals will understand the roles and responsibilities of individual members as part of the healthcare team, including their ability to promote the delivery of quality healthcare. They will interact effectively and sensitively with all members of the healthcare team.

8.2 Team Member Participation

8.23 Apply effective techniques for managing team conflict.

Foundation Standard 11: Information Technology Applications

Healthcare professionals will use information technology applications required within all career specialties. They will demonstrate use as appropriate to healthcare applications.

11.3 Basic Computer Literacy Skills

11.31 Apply basic computer concepts and terminology in order to use computers and other mobile devices.

11.32 Demonstrate basic computer operating procedures.

11.34 Use basic work processing, spreadsheet, and database applications.

11.35 Evaluate the validity of web-based resources.

11.36 Demonstrate use of appropriate email and social media usage.

Michigan Career & Technical Education Standards (26.0102 Biotechnology Medical Sciences)

Technical Standards

I. Academic Foundation

A. Human Structure and Function

1. Classify the basic structural and functional organization of the human body including chemical, cellular, tissue, organ, and system

VII. Safety Practices

D. Common Safety Hazards

2. Comply with safety signs, symbols, and labels

3. Understand implications of hazardous materials

4. Apply safety principles within given environments

VIII. Teamwork

B. Team Member Participation

2. Respect and value the expertise and contributions of all team members

5. Apply effective techniques for handling team conflict

XI. Information Technology Applications

B. Information Technology

1. Implement communications using technology (i.e. Fax, E-mail, and Internet) to access and distribute data and other information

2. Execute the use of software, hardware, and the Internet

Pathway Standards

II. Academic Foundations

A. Biotechnology R&D professional will be knowledgeable in the fundamentals of biochemistry, cell biology, genetics, mathematical concepts, microbiology, molecular biology, organic chemistry, and statistics

3. Understand Genetics

a. Describe the basic structure of a chromosome

6. Apply principles of cell biology

a. Describe the basic structures and functions of cells and how this knowledge is used in biotechnology

b. Select cellular barriers to be overcome for a biotechnology product to work inside a cell

7. Apply principles of molecular biology

a. Diagram the structure of the nucleic acid DNA

b. Demonstrate DNA replication graphically and its importance to biotechnology product development

IV. Laboratory Protocols and Procedures

A. Biotechnology R&D professionals will understand the principles of solution preparation, sterile techniques, contamination control, & measurement & calibration of instruments. They will maintain a safe laboratory environment using biosafety protocols.

2. Apply protocols

a. Describe the criticality of the requirement of sterile technique

Career & Employability Standards

I. Applied Academic Skills

C. Listening & Presentation Skills

1. Use correct grammar to communicate verbally

V. Personal Management

B. Self-Management

1. Monitor & evaluate accurately one's progress towards a goal or completion of a project

4. Prioritize and accomplish tasks independently

VI. Organizing Skills

C. Materials

3. Acquire resources in a timely fashion and take responsibility for their care

4. Identify and prepare tools, equipment, space, and facilities appropriate for a task

5. Work within constraints of safety precautions and available resources

D. Human Resources

1. Learn cooperation and leadership in a team at school or in a workplace setting

2. Organize and communicate with members of a team using varied methods of communication

4. Acknowledge and utilize the skills, abilities, and input of all members of a team

VII. Teamwork

A. Group Participation

2. Take personal responsibility for influencing and accomplishing group goals

XI. Technology Skills

C. Technology Productivity Tools

4. Apply advanced software features such as an application's built-in thesaurus, templates, and styles to improve the appearance of work processing documents, spreadsheets, and presentations

5. Identify technology tools (e.g., authoring tools or other hardware or software resources) that could be used to create a group project

8. Use a variety of applications to plan, create, and edit a multimedia product (e.g., model, webcast, presentation, publication, or other creative work)

Materials & Equipment Checklist

Lesson 1 Electrophoresis & Genetic Trait Exploration

- Dye Gel Electrophoresis
  - Provided by kit (*Biotechnology Explorer, STEM Electrophoresis Kit, 166-5080EDU, Bio-Rad*):
    - Dye extraction solution, 25 ml, 1 bottle
    - Blue 1 reference dye, 150 µl, 1 vial
    - Yellow 5 reference dye, 150 µl, 1 vial
    - Yellow 6 reference dye, 150 µl, 1 vial
    - Red 40 reference dye, 150 µl, 1 vial
    - Electrophoresis buffer, 50x TAE, 100 ml, 1 bottle
    - Molecular biology grade agarose, 5 g, 1 bottle
    - 2 ml microcentrifuge tubes, 72 tubes
    - Hinged plastic boxes, 1 box
    - Paper clips, 4
    - Black alligator clip leads, 2
    - Red alligator clips leads, 2
    - 8-well combs, 2
  - Not provided by kit:
    - 9 V batteries, 6–10
    - Plastic rulers or plastic card to cut gels, 2
    - 2–20 µl adjustable-volume micropipette or 10 µl fixed-volume micropipette, 2
    - 2–200 µl pipet tips, 1,000/bag, 1 bag
    - Eyedroppers or 100–1,000 µl adjustable-volume micropipette, 2; or disposable plastic transfer pipettes (DPTPs), 1 box; or 100–1,000 µl pipet tips, 1 bag
    - Marking pen, 2
    - Plastic cups or small beakers, 8
    - Microwave oven or hot plate, 1
    - Balance, 1
    - Distilled water, 1 liter
    - 500 ml Erlenmeyer flask for microwaving agarose, 1
    - Candies with a variety of color coatings variable (M&M's, Skittles, or Kool-Aid drink mixes)
  - Optional:
    - Microcentrifuge or mini centrifuge, 1
    - Digital camera for imaging gels, 1
    - Microcentrifuge tube racks, 8

Lesson 2 Dye Electrophoresis & Gene Exercises

- Dye Gel Electrophoresis (see list given for previous page)

Lesson 3 Class Discussion of Electrophoresis and Genetic Traits

Lesson 4 Introduction to Human *Alu* PV92 & Isolation of Cheek Cell DNA

- Isolation of Cheek Cell DNA
    - Provided by kit (*Biotechnology Explorer, Chromosome 16: PV92 PCR Informatics Kit, 166-2100EDU, Bio-Rad*):
        - 1.5 ml Micro test tubes, with attached caps
        - Screw cap tubes with 200 µl InstaGene matrix
        - Foam micro test tube holders
    - Not provided by kit:
        - P-20 micropipet
        - Pipet tips (filter type), 2-20 µl
        - Permanent marker
        - Biological waste container
        - Cups with 10 ml 0.9% saline
        - P-1000 or P-200 micropipet
        - Pipet tips (filter type), 100-1000 µl or 20-200 µl
        - Water baths (56 and 100 °C)
        - Microcentrifuge or mini centrifuge
        - Vortexer

Lesson 5 PCR Amplification of DNA

- PCR Amplification of DNA
    - Provided by kit (*Biotechnology Explorer, Chromosome 16: PV92 PCR Informatics Kit, 166-2100EDU, Bio-Rad*):
        - PCR tubes
        - 1.5 ml Micro test tubes, capless
        - Complete master mix (containing primers) on ice
        - Foam micro test tube holders
        - Molten agarose
    - Not provided by kit:
        - P-20 micropipet
        - Pipet tips (filter type), 2-20 µl
        - Ice bucket with ice
        - Permanent marker
        - Biological waste container
        - Gel trays
        - Lab tape for gel trays
        - Microcentrifuge or mini centrifuge
        - Thermal Cycler

Lesson 6 DNA Analysis by Gel Electrophoresis

- DNA Analysis by Gel Electrophoresis

- Provided by kit (*Biotechnology Explorer, Chromosome 16: PV92 PCR Informatics Kit, 166-2100EDU, Bio-Rad*):
  - PV92 XC DNA loading dye
  - EZ Load molecular mass ruler (DNA standards)
  - Foam micro test tube holders
  - Gel staining tray
  - Fast Blast DNA stain, 1x or 100x solution
  - 1x TAE electrophoresis buffer
  - Amplified positive control samples
    - PV92 homozygous (+/+)
    - PV92 homozygous (-/-)
    - PV92 heterozygous (+/-)
- Not provided by kit:
  - Prepared agarose gel
  - Prepared PCR samples
  - P-20 micropipet
  - Pipet tips (filter type), 2-20 μl
  - Permanent marker
  - Gel box and power supply
  - Gel support film (optional)
  - Clear acetate sheets for tracing gels (optional)
  - Warm tap water for detaining gels (if performing quick staining protocol)
  - Large containers for destaining (if performing quick staining protocol)
  - Biological waste container
  - Rocking platform (optional)
  - Microcentrifuge or mini centrifuge

Lesson 7 Analysis and Interpretation of Your Results

Lesson 8 Molecular Techniques and Genetic Diseases/Disorders

Lesson 9 Pharmacogenomics

Lesson 10 Introduction to Genetic Based Diseases/Disorders

Lesson 11 Genetic Disorder/Disease Presentations

Notes:

Biotechnology Explorer, STEM Electrophoresis Kit, 166-5080EDU, Bio-Rad

Kit contains sufficient materials for 2 student workstations.

Biotechnology Explorer, Chromosome 16: PV92 PCR Informatics Kit, 166-2100EDU, Bio-Rad

Kit contains sufficient materials for 8 student workstations, with 4 students at each station.

Lesson Schedule

Week 1

Monday	Lesson 1: Electrophoresis & Genetic Trait Exploration
Tuesday	Lesson 2: Dye Electrophoresis & Gene Exercises
Wednesday	Lesson 3: Class Discussion of Electrophoresis and Genetic Traits
Thursday	Lesson 4: Introduction to Human *Alu* PV92 & isolation of Cheek Cell DNA
Friday	Lesson 5: PCR Amplification of DNA

Week 2

Monday	Lesson 6: DNA Analysis by Gel Electrophoresis
Tuesday	Lesson 7: Analysis and Interpretation of Your Results
Wednesday	Lesson 8: Molecular Techniques and Genetic Diseases/Disorders
Thursday	Lesson 9: Introduction to Genetic Based Diseases/Disorders
Friday	Lesson 10: Pharmacogenomics

Week 3

Recommendation: Give students 2-3 workdays so they have in-class time to work with their groups on the presentations.

Thursday	Lesson 11: Genetic Disorder/Disease Presentations – Day 1
Friday	Lesson 11: Genetic Disorder/Disease Presentations – Day 2 (if needed)

Take Home Tasks for Students

Week 1

Monday Lesson 1: Electrophoresis & Genetic Trait Exploration

Complete: "Determining Your Procedure"

Homework: "Human Genetic Traits Inventory", "Human Karyotype Exercise"

Tuesday Lesson 2: Dye Electrophoresis & Gene Exercises

Homework: "Genetic Traits Video Questions"

Review: agarose, buffer, wells, electrodes, pipet, gel chamber, comb, centrifuge

Wednesday Lesson 3: Class Discussion of Electrophoresis and Genetic Traits

Review: DNA, Central Dogma, Genes, Heredity, Electrophoresis

Thursday Lesson 4: Introduction to Human *Alu* PV92 & isolation of Cheek Cell DNA

Complete: "Template Preparation Discussion Questions"

Review: Alu PV92, intron, exon, noncoding DNA, homozygous, heterozygous, isolation

Friday Lesson 5: PCR Amplification of DNA

Complete: "Understanding PCR" Exercise

Review: PCR, complementary strand hybridization, strand synthesis, DNA polymerase, deoxynucleotides, primers, denature, anneal, extend, thermal cycler, supernatant

Week 2

Monday Lesson 6: DNA Analysis by Gel Electrophoresis

Complete: "Discussion Questions"

Review: loading dye, electrodes, current, buffer, staining

Tuesday Lesson 7: Analysis and Interpretation of Your Results

Complete: "Interpreting Your Gel"

Wednesday Lesson 8: Molecular Techniques and Genetic Diseases/Disorders

Complete: "Investigating Genetic Disorders"

Thursday Lesson 9: Introduction to Genetic Based Diseases/Disorders

Complete: "Genetic Disorders"

Friday Lesson 10: Pharmacogenomics

Complete: "Pharmacogenomics Exploration"

Homework: Select a genetic disease/disorder and begin collecting information

Week 3

Recommendation: Give students 2-3 workdays so they have in-class time to work with their groups on the presentations.

Thursday Lesson 11: Genetic Disorder/Disease Presentations – Day 1

Friday Lesson 11: Genetic Disorder/Disease Presentations – Day 2 (if needed)

Advance Laboratory Preparation Schedule

Instructor's Lesson Plan
Advanced Preparation and Instruction

Lesson 1 Electrophoresis & Genetic Trait Exploration

Advanced Preparation

Prepare molten agarose (15 min)

Materials Needed for Advanced Preparation	Quantity
Electrophoresis buffer, 50x TAE	25 ml
Molecular biology grade agarose	5 g
Marking pen	1
Balance	1
Erlenmeyer flask	1
Microwave oven or hot plate	1

The recommended agarose concentration for gels in this application is 1% agarose. This concentration of agarose provides good resolution and minimizes run time required for electrophoretic separation of the dyes. The volume required for each gel is 50 ml. This volume of agarose ensures that the wells are deep enough to easily pipet 10 μl of sample into each well and that there is space above the gel to overlay with running buffer. **Be sure to use 1x TAE electrophoresis buffer, not water, to prepare agarose gels**.

1x TAE electrophoresis buffer preparation

TAE (Tris-acetate-EDTA) electrophoresis buffer is provided as a 50x concentrated solution. In addition to the 1x TAE buffer needed to prepare the agarose gels, 55 ml of buffer is also required for each electrophoresis chamber. One liter of 1x TAE buffer will be sufficient to prepare and run 8 agarose gels. To make 1 L of 1x TAE buffer from 50x TAE concentrate, add 20 ml of 50x concentrate to 980 ml of distilled water and mix. Aliquot 50 ml of buffer into smaller Erlenmeyer flasks for student use.

Agarose preparation

These procedures may be carried out 1 to 2 days ahead of time by the teacher. The instructions below are for the preparation of the molten agarose assuming the students will prepare their own gel boxes and pour their own gels.

i) To make 1% agarose solution, use 1 g of agarose for 100 ml of 1x TAE electrophoresis buffer. Be sure to use electrophoresis buffer, not water.

Use the following guide for gel volume requirements when casting single or multiple gels.

Number of gels	Volume of 1% agarose
1	50 ml
2	100 ml
4	200 ml
8	400 ml

ii) Add the agarose powder to a suitable container (for example, use a 500 ml Erlenmeyer flask for 200 ml or less). Add the appropriate amount of 1x TAE electrophoresis buffer. If clumps of agarose are visible, swirl the flask to mix.

iii) While heating the solution, some of the volume will be lost to evaporation. Prior to heating, mark the volume level on the flask or weigh the flask and content and record the weight.

iv) Dissolve the agarose by heating in a microwave oven. If a microwave is not available, the solution can be heated on a magnetic hot plate.

Caution: Always wear protective gloves, goggles, and lab coat while preparing and casting gels. Molten agarose or the flasks containing hot agarose can cause severe burns if allowed to contact skin.

a. Microwave oven method

This technique is the fastest and safest way to dissolve agarose. Place the gel solution in an appropriate bottle or flask into the microwave. If you are using a bottle, **be sure to loosen the cap before heating**. The ideal microwave setting will depend on the volume of agarose solution that you are preparing and on the power of the microwave oven. For small volumes, microwave solution for 1 min, swirl the solution, then microwave in 20-30 sec intervals, swirling after each, until **all** of the small transparent agarose particles are dissolved. For larger volumes (e.g. 400 ml), heat initially for 2-3 min, swirl the solution, then microwave in 30-45 sec intervals, swirling after each, until **all** of the small transparent agarose particles are dissolved.

Add water to the agarose solution to bring it back to the original volume and swirl to mix completely. Cool agarose solution to 55-60 °C before pouring gels. If students are going to pour their own gels, keep the agarose at 55-60 °C until it is ready to be poured by either stirring on a hot plate or in a water bath. If the agarose is kept warm in a water bath, make sure to swirl it thoroughly to mix the solution immediately before the gels are poured.

b. Magnetic hot plate method

Add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Boil the solution until **all** of the small transparent agarose particles are dissolved.

Add water to the agarose solution to bring it to the original volume and swirl to mix completely. Cool agarose gels to 55-60 °C before pouring gels. If students are going to pour their own gels, keep the agarose at 55-60 °C until it is ready to be poured by stirring on a hotplate. Aliquot 50 ml of molten agarose into smaller Erlenmeyer flasks for student use.

Aliquot dye extraction solution and reference dyes (45 min)

Aliquot dye extraction solution

Materials Needed for Advance Preparation	**Quantity**
Dye extraction solution	25 ml
2 ml Microcentrifuge tubes	8
Marking pen	1
100-1,000 µl adjustable-volume micropipette and tips or DPTPs	1
Candies	

Label eight clean 2 ml Microcentrifuge tubes **Extraction** and aliquot 2 ml of dye extraction solution into each tube.

Aliquot reference dyes

Materials Needed for Advance Preparation	**Quantity**
Blue 1 reference dye	150 µl
Yellow 5 reference dye	150 µl
Yellow 6 reference dye	150 µl
Red 40 reference dye	150 µl
2 ml Microcentrifuge tubes	32
2-20 µl adjustable-volume micropipette and tips	1
Marking pen	1

1. If you have a centrifuge, pulse spin the reference dyes to pool the solutions at the bottom of the tubes.

2. Aliquot Blue 1 reference dye

   Label eight clean 2 ml Microcentrifuge tubes **Blue 1** and aliquot 15 µl of Blue 1 reference dye into each tube.

3. Aliquot Yellow 5 reference dye

   Label eight clean 2 ml Microcentrifuge tubes **Yellow 5** and aliquot 15 µl of Yellow 5 reference dye into each tube.

4. Aliquot Yellow 6 reference dye

   Label eight clean 2 ml Microcentrifuge tubes **Yellow 6** and aliquot 15 µl of Yellow 6 reference dye into each tube.

5. Aliquot Red 40 reference dye

   Label eight clean 2 ml Microcentrifuge tubes **Red 40** and aliquot 15 µl of Red 40 reference dye into each tube.

Set up workstations (30 min)

The kit provides enough materials for 8 student workstations. Workstations should include no more than 4 or 5 students. Carefully ensure that each workstation is equipped with the following materials.

Student Workstation	**Quantity**
Plastic chamber	1
8-well comb	1
Ruler	1
Molten agarose	50 ml
Marking pen	1
Paper clips	2
Black lead with alligator clips	1
Red lead with alligator clips	1
9 volt batteries	3-5
Blue 1 reference dye	15 µl
Yellow 5 reference dye	15 µl
Yellow 6 reference dye	15 µl
Red 40 reference dye	15 µl
1x TAE buffer	22 ml
Dyes extracted from candies	4 samples
2-20 ml adjustable-volume micropipette and tips	1

Lesson 1 Electrophoresis & Genetic Trait Exploration

Instruction

The students should come into class where their workstations are already set up and "Identifying the Components" and "Determining Your Procedure" has been printed out for them, and are both waiting at their seat.

10 minutes Instructions

Explain that students will spend time today freely exploring agarose gel electrophoresis. They should be sure they understand the different components (Identifying the Components) needed to run such a procedure. Once they have explored the various individual components, encourage them to try setting one up and running it. While they are exploring how to run a gel, they should come up with the steps needed to run a gel in their own words (Determining the Procedure).

30 minutes Activity

The instructor should move about the classroom, interacting with students to be sure learning takes place throughout the class period. Offer assistance or ask students questions regarding the task to keep them motivated. Explore concepts such as how the agarose gel works to separate DNA, what the buffer does for the process, and why students should be aware of where the (+) and (-) electrodes are located. Allow them, as much as possible, to come to their own conclusions. Use a Socratic method of instruction to help them defend their ideas for the procedure.

20 minutes Wrap-Up

Ask the students to turn in their worksheets ("Identifying the Components" and "Determining Your Procedure") before they leave. The instructor can look these over and use them to identify points of discussion during the next day's lesson.

Introduce the homework; "Human Genetic Traits Inventory" and "Human Karyotype Exercise". Review terminology for both worksheets (allele, gene, dominant, recessive, genotype, and phenotype). Work through the first item on the Human Genetic Traits Inventory (tongue rolling) as a class so the students have an example to work from. Explain the nomenclature (RR, Rr, and rr). Remind them that if they know they are a dominant phenotype, but are unsure of their genotype, they may answer R_. Review concepts related to the Human Karyotype Exercise such as those described on the first page (chromosome length, centromere position, banding pattern, and satellite endings). These two worksheets will be due by the day of the third lesson.

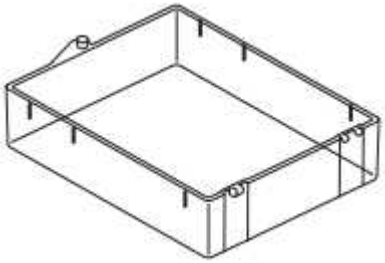
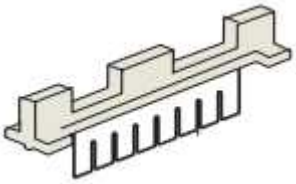
Lesson 1 Electrophoresis & Genetic Trait Exploration

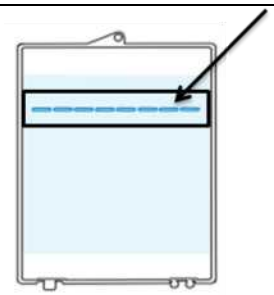
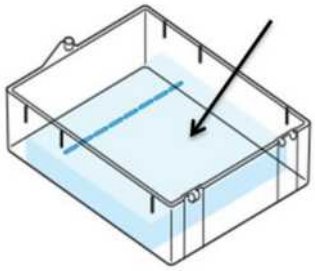
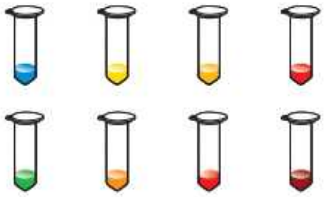
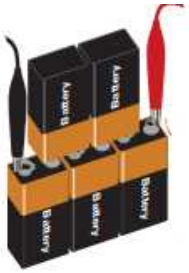
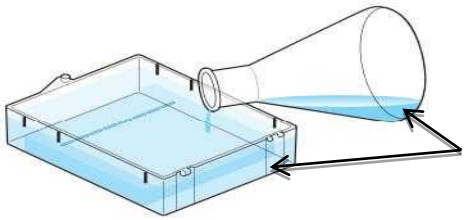
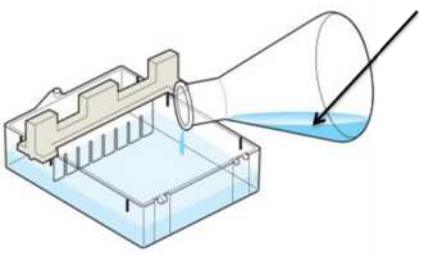
Identifying the Components

Agarose gel electrophoresis is a method that uses electrical current to separate macromolecules, such as DNA, RNA, and proteins, and their fragments based on their size and charge. Before you can use this technique, though, you need to know what materials you'll be working with during the lab. Take a look at the components, both materials and equipment, in front of you on your lab bench and at the shared table for the lab. Is there anything you recognize right away? What are some things you have never seen before? Can you work with your lab mates to identify the different components and take a guess at what they might be used for during agarose gel electrophoresis? Below are some helpful pictures and names for the different components you might encounter. See if you can match any of them with one another.

Word Bank		
Wells	**Solidified Agarose Gel**	**Leads**
Buffer	**Pipet Tip**	**Comb**
Microcentrifuge Tubes	**Molten Agarose Gel**	**Centrifuge**
Electrodes	**Samples (Known and Unknown)**	**Pipet**
Power Supply		**Gel Electrophoresis Chamber**

Select words from the word bank above and fill in spaces A-N to identify the pictures below them.

A: **Gel Electrophoresis Chamber**	B: **Comb**	C: **Centrifuge**
		

D: **Wells**	E: **Electrodes**	F: **Solidified Agarose Gel**
		
G: **Samples (Known and Unknown)**	H: **Pipet** I: **Pipet Tip**	J: **Power Supply**
		
K: **Leads**	L: **Buffer**	
		
M: **Microcentrifuge Tubes**	N: **Molten Agarose Gel**	
		

Lesson 1 Electrophoresis & Genetic Trait Exploration

Determining Your Procedure

Now that you are familiar with the different components with which you will be working, how should you move forward with your agarose gel electrophoresis procedure? What are some things you should consider related to your sample, putting together the electrophoresis apparatus, and making and preparing your gel for samples? How long do you think the gel should be run and how will you know when it is done? What might you need to do with the gel once the samples are done running? Using the space below and on the back of this page, work with your lab mates to number and describe several steps, in logical order, you would use to complete this procedure.

Suggested procedure (do not share with students):

1. **Measure and mark a distance of 1 cm, on the outside of the box, from both ends of the box on both the longest sides with a marker.**
2. **Measure and mark a distance of 3 cm on both the longest sides from one end of the box with a marker.**
3. **Place the comb on the 3 cm mark and be sure it is centered in the box so none of the combs are touching the plastic box.**
4. **Prepare 50 ml of molten agarose and gently pour it into the box. Wait 10-20 minutes for the gel to solidify.**
5. **Carefully remove the comb from the solidified gel.**
6. **Cut the gel at each of the 1 cm marks and remove the ends of the gel from the box.**
7. **Prepare and add 55 ml of 1x TAE buffer to the box.**
8. **Construct the electrodes by bending the paperclips until they make a "U" shape and insert one at each end of the box with the arms sticking straight up out of the buffer.**
9. **Prepare the extracted dye samples, and using a separate tip for each color, load 10 μl of each sample, one into each of the 8 wells.**
10. **Assemble the battery tower by connecting the negative nodes to positive nodes.**
11. **Attach the black alligator clip to the long end of the paper clip at the end of the box closest to the wells, and attach the red alligator clip to the long end of the paper clip at the other end of the box.**

12. **Attach the other end of the alligator clips to the positive and negative terminals, respectively, on the battery tower.**

13. **Allow the gel to run about 20 minutes, but disconnect the alligator clips from the battery tower before the dyes run off the end of the gel.**

14. **Take a picture of the gel to record the results.**

Notes:

Students will not normally know the volume needed for each of the reagents, which is fine at this point. They may also not understand the need to cut the gel or the role the paper clips play. Guide their understanding toward the idea that the buffer is the conductor for the electrical current from the batteries, and it is this current which allows the samples to move down the gel. The gel must be cut so that the buffer can surround the gel adequately. The paperclips act as the electrodes to help transmit this current. Ask them, "What causes the samples to move through the gel?" Ask, "What role do you think the batteries play in this procedure?" Introduce them to the idea of how samples separate by size on the gel by asking, "Why do some samples move further than others?" or "How is it that the larger sized samples will stay closer to the wells while the smaller samples will be found toward the end of the gel?" Students typically understand the need for and purpose of the comb well. Engage students in conversation regarding contamination and the importance of using separate tips for each sample. Also, ask them questions about how to know when to stop their gels. This will be an opportunity to introduce the ideas of a loading dye. The preparation of each of the reagents will also be discussed in more detail at later points in this exercise. There will also be an opportunity to review proper pipetting skills later as well.

Safety:

For the most part, there are no sever hazards with this laboratory procedure. Students should be reminded to follow standard laboratory safety procedures (do not smell or taste anything at the lab bench and wear gloves, goggles, and a lab coat if available). The most critical part to be aware of is the temperature of the molten agarose. Students to take caution in preparing and pouring the molten agarose because it will be hot and could cause severe burns if allowed to contact skin.

Otherwise, this is the opportunity for students to make mistakes and learn from them.

Lesson 1 Electrophoresis & Genetic Trait Exploration

Human Genetic Traits Example Answers

After reviewing the phenotypes on the previous page, complete the inventory below by determining your own phenotype and genotype as best you can. You may need to think about the traits in your parents to help determine the details. We will then take a look at the total inventory for our class when everyone is complete.

Trait	Symbol	Dominant Phenotype	Your Phenotype	Your Genotype	Number of Phenotypes in Class*	
					Dominant	Recessive
1. Tongue Rolling	R, r	Rolled	R_	Rolled	5	5
2. Facial Dimples	D, d	Present	dd	Not Present	7	3
3. Bent Little Finger	B, b	Bent	bb	Not Bent	8	2
4. Free Ear Lobes	F, f	Free	F_	Free	4	6
5. Mid-digital Hair	H, h	Present	H_	Present	1	9
6. Hand Clasping	L, l	Top Left Thumb	L_	Top Left Thumb	3	7
7. Widow's Peak	W, w	Present	ww	Not Present	2	8
8. Hitchhiker's Thumb	T, t	Absent	T_	Absent	4	6
9. Cleft Chin	C, c	Present	cc	Not Present	9	1
10. Eye Color	M, m	Melanin (Brown)	M_	Melanin (Brown)	5	5

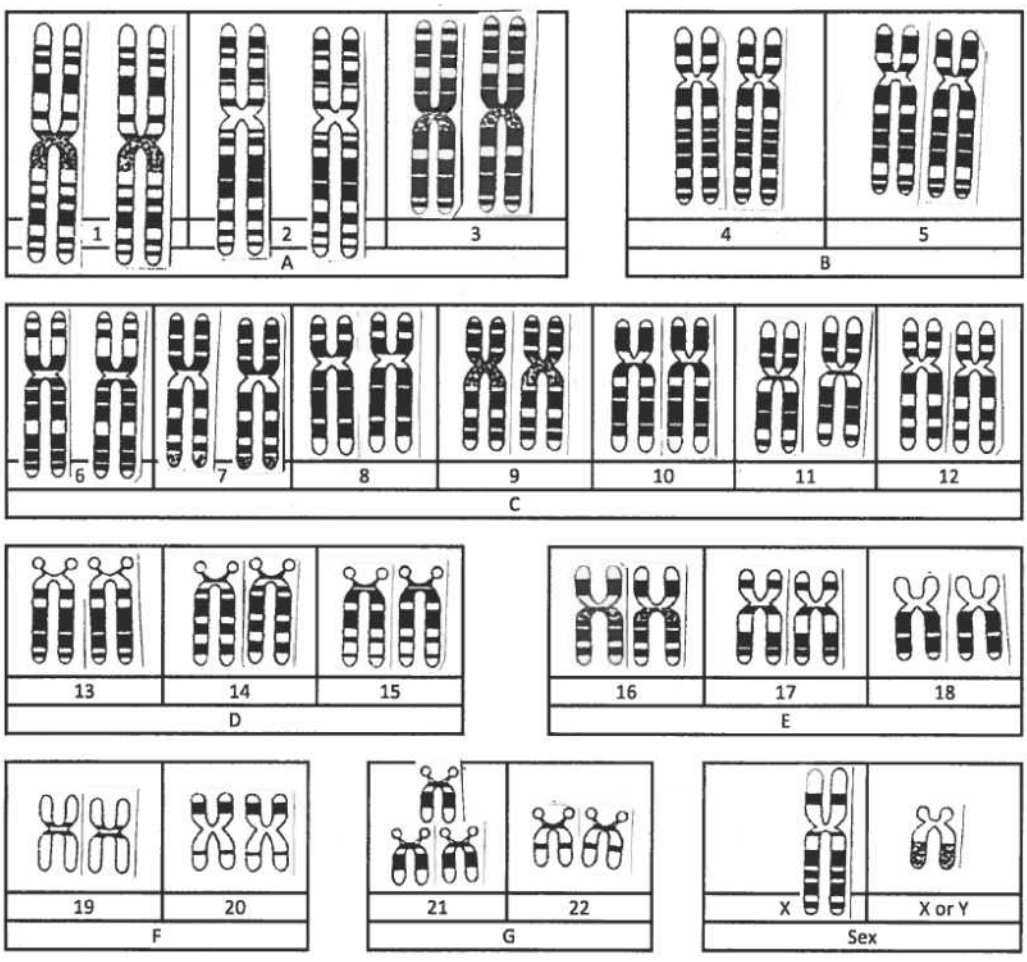
** This is sample data. Actual class will yield different results.

Lesson 1 Electrophoresis & Genetic Trait Exploration

Human Karyotype Exercise Answers

For this exercise, you will be working with a partner to create your own karyotype. From the chromosome spread on the next sheet, carefully cut out each chromosome. Find the homologous pairs by matching length, the position of the centromere, and the banding pattern. Arrange the pairs on the karyotype form below in order from longest to shortest, placing the sex chromosomes at the end. Lightly tape the chromosomes in place. Once you are confident that you have them placed correctly, tape them down more securely. Then answer the following questions:

a) What is the sex of this individual: **Male** or Female ? (circle one)
b) Determine the state of the chromosomal arrangement: Normal or **Abnormal** ? (circle one)
c) If the arrangement is abnormal, state in what way: ___**Trisomy 21_(Down Syndrome)**_____



Lesson 2 Dye Electrophoresis & Gene Exercises

Advanced Preparation

Refer to the **Advanced Preparation** for Lesson 1 located on pages 26-29.

Lesson 2 Dye Electrophoresis & Gene Exercises

Instruction

The students should come into class where their workstations are already set up and the rest of the unit workbook is printed and available for them.

10 minutes Instructions

Explain to the students that they will actually be running a demonstration gel today using dyes extracted from candies (rather than DNA). It will be their job to determine the dye in their 'unknown' when compared to the four standards that are provided. Ask students to form a prediction regarding their 'unknown'. Do students think all the dyes will move at the same rate? (There may be variability due to size and charge). Will they travel the same distance? (There will be variability due to size). What might cause some of the differences they might see? (Some dyes are larger than others or have a higher charge). How do they think their unknown will compare to the reference dyes? Do students think all the dyes will remain in one band? (Some of the candies may be colored with a combination of dyes).

Review the various reagents and equipment, making reference to the items they explored in the previous lesson.

40 minutes Activity

Begin this part of the lesson by prompting students with questions regarding the procedure such as; why do you have to put the comb in before pouring the agarose? (To avoid adding air bubbles to the agarose; this will impede the movement of samples through the gel.) Why do you need to add buffer to run the gel? (The buffer provides a medium for the electric current.) Why do the colored candy solutions have to be centrifuged? When will you know your gel is done running? Students should start by preparing the gel box. Once all groups have reached this point, and are waiting for their agarose to solidify, have them work on the dye extraction from candies.

When the agarose has solidified, students can prepare the gel, as indicated in the procedure, to run their candy samples. **Be sure they spin down their samples to bring all the liquid to the bottom of their microcentrifuge tubes.**

If students are less experienced with pipets, you may want to give a brief demonstration showing how to successfully load the samples into the wells of the gel, pointing out that it is important that they not go too far in and puncture the well. Steadying their elbows on the lab bench and using both hands is the best approach. **Emphasize the importance of using a different tip for each sample.**

Once samples are loaded and the power supply is connected, students should watch to be sure their samples start moving through the gel in the correct direction. If there is enough class time, students can monitor their gels until they finish running, and photograph the results.

10 minutes Wrap-Up

If there is not enough class time, inform the students that you will monitor their gels as they finish running, and take photographs for them to see in the next class period.

Introduce the homework; "23andMe.com Video Questions". Explain that this assignment will build on the homework from the previous lesson regarding genetics and heredity. They will need to watch the videos and answer the questions. Along with the homework form lesson 1, this assignment will be reviewed in lesson 3.

Lesson 2 Dye Electrophoresis & Gene Exercises

Homework: Genetic Traits Video Questions

(Adapted from 23andMe.com)

- Visit the following website: www.23andMe.com/gen101/
- Watch the following videos and answer the questions below: What Are Genes? What are SNPs? Where Do Your Genes Come From? What Are Phenotypes?

What Are Genes?

1. Where do most of the genes reside in your body? __**cell nucleus**_____
2. "DNA is a double-stranded molecule composed of ____**sugar**_____, ___**phosphate**_____, and four different bases; ____**adenine**_____, ___**thymine**_____, _____**cytosine**_____, and ____**guanine**_____."
3. "Most genes are recipes for making specific _____**proteins**_____."
4. "Gene _____**regulators**_____ turn different genes on and off in different cells to control cell function."
5. How many pairs of chromosomes do humans have? ____**23**_____
6. What percentage of the DNA in your chromosomes do you share with chimpanzees? _____**98.5%**_____ . Other humans? _____**99.5%**_____

What Are SNPs?

1. An entire set of 23 human chromosomes is called a ____**genome**_____.
2. Variation at a single base pair is called a SNP, or ____**single**_____ ____**nucleotide**_____ ____**polymorphism**_____.
3. An SNP is created when a single base pair is ____**left out**_____, _____**added**_____, or ____**substituted**_____.

4. **True** or False. SNPs account for the genetic variation between you and other humans.
5. **True** or False. Many SNPs lead to no observable differences between people at all.
6. **True** or False. SNPs can be used to trace ancestry.

Where do Your Genes come from?

1. You inherit one ____**set**_____ of chromosomes from each _____**parent**_____, which together are called ____**homologous**_____ chromosomes.
2. Most cells contain two sets of chromosomes, but ____**sperm**_____ and _____**egg**_____ cells each contain only one set of chromosomes.
3. Your siblings also receive one set of genes from each of your parents, but not necessarily the same combination of genes unless you are ____**identical**_____ _____**twins**_____.
4. When homologous chromosomes exchange DNA by crossing over, this is called genetic ____**recombination**_____.
5. Ancestry along your father's line is easier to trace through the ____**y chromosome**_____, and through your mother's line it is easier to trace through the ____**mitochondrial DNA**____.

What Are Phenotypes?

1. Your _____**observable**_____ _____**traits**_____, also called your phenotype, results from the interaction between your genes and the environment.
2. Give two examples of phenotypes which are controlled by the interaction between your genes and the environment, such as diet and exercise; 1) _____**height** _____and 2) _____**body weight**_____.
3. **True** or False. Not much is known about how your genes affect your personality.

Lesson 3 Class Discussion of Electrophoresis and Genetic Trait

Instruction

Students should come in with the homework from lesson 1 ('Human Genetic Traits Inventory" and "Human Karyotype Exercise") and lesson 2 ("23andMe.com Video Questions") complete. If there was not enough time in class during the previous lesson, they should also come in to class to find a photograph of their gel at their workstations. To prepare, draw a chart on the board similar to the answer sheet for "Human Genetic Traits Inventory".

20 minutes Review genes and heredity

Ask students to go up to the board and put tally marks representing their phenotype (dominant or recessive) for the 10 characteristics listed in the "Human Genetic Traits Inventory". Review the results. Ask the students if they notice anything interesting; was it difficult to determine some of their phenotypes or genotypes, were they able to know their genotypes based on family information, are there phenotypes that the majority of the class shares? Ask questions such as; if a gene is recessive, does that mean fewer people will have it? What data from our class supports your answer?

Review the "Human Karyotype Exercise" questions. Have the students compare their results with a neighbor. Ask them what was easy about this exercise, and what was difficult. Explain that this is just one way scientists or doctors could look for abnormalities in genes.

Do a round-robin to check the answers on the "23andMe.com Video Questions" worksheet. Check at each section for concepts that were difficult or confusing to students and offer clarification.

As a review, and to further reinforce concepts, employ a class discussion to find answers for the questions asked on the "Review of DNA and Genes" worksheet. Make connections to the idea that one must understand the structure and mechanism by which DNA works in order to know how to extract information from it such as with karyotyping and electrophoresis.

20 minutes Review of dye electrophoresis

Allow the students the time to review the photographs of their gels from the previous lesson. They will use "Introduction to Electrophoresis" to review their results. Students should spend about 10 minutes working through the questions on this worksheet with their groups. The instructor should move about the room to survey the discussion and make sure students are on task and writing down answers to all of the questions. If students appear to be stuck, instructors may want to ask a question to help point them

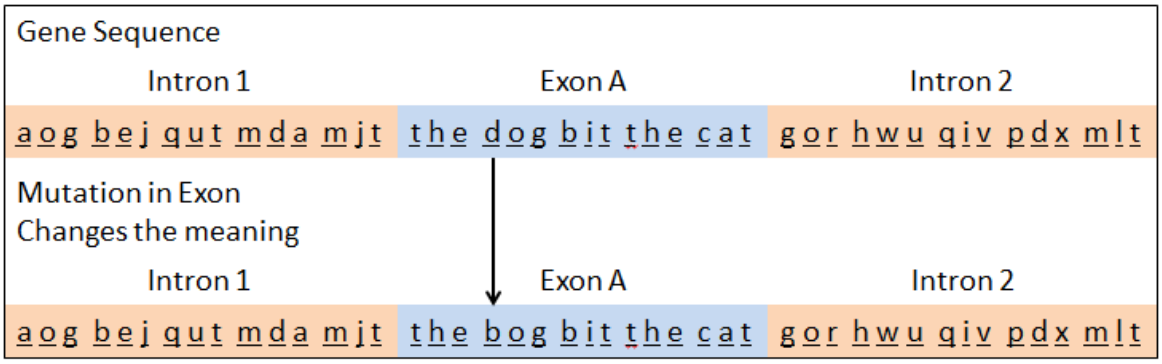
in the right direction but avoid giving answers. Encourage the discussion with questions such as what causes the sample to move through the gel? Why do the samples move at different rates? Emphasize the relationship between the various concepts. Why might a mother and a child have different results in an electrophoresis of the same gene region?

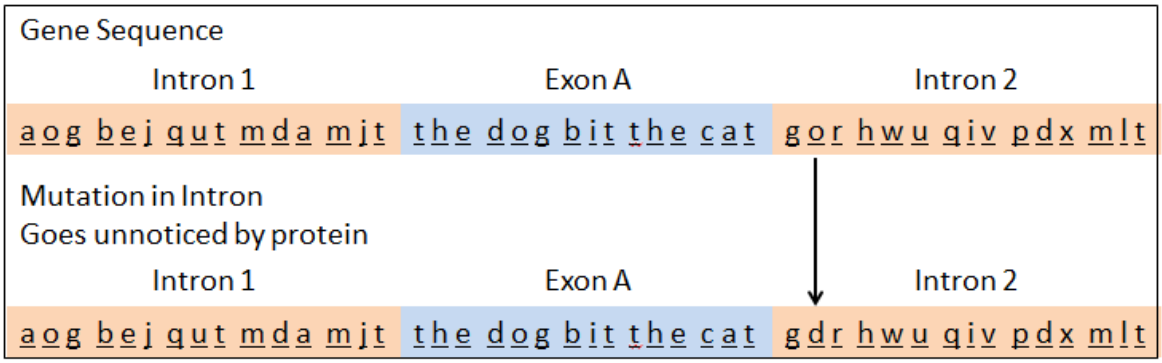
The goal for instruction is to get the students to make connections and understand how these concepts can be applied to solve larger problems. Once most groups have completed their sheets, have students share their responses with the class being sure to elicit answers from different groups. Also, through discussion, explore how their procedures (from lesson 1) differ from the textbook procedure given to them in lesson 2. Understand why the differences exist and be sure they understand the necessity and order of the steps given in the textbook procedure. Discuss the purpose of each step (assembling the gel chamber, preparing the gel, preparing the samples, loading the gel, running the gel, analyzing the results).

20 minutes DNA processing

Explain to students that not all mutations will be noticeable in a person's phenotype such as was investigated in the "Human Genetic Traits Inventory" or even visible in a karyotype. Demonstrate the processes of transcription and translation; from genotype to phenotype using the scaffold at the end of the "Introduction to Electrophoresis" worksheet. Help illustrate this process by showing the class the video located at: http://www.yourgenome.org/teachers/dnaprotein.shtml. Explain that some mutations will impact a person's phenotype (if they occur in the exon region), while others may go unnoticed (if they are in the intron region). Those that go unnoticed in the phenotype may only cause a difference in the genetic code. This difference may be detectable through applications such as agarose gel electrophoresis.

Analogy for mutations (for classroom instruction):


Gene Sequence
Intron 1
Exon A
Intron 2
aog bej qut mda mjt
the dog bit the cat
gor hwu qiv pdx mlt
Mutation in Exon
Changes the meaning
Intron 1
Exon A
Intron 2
aog bej qut mda mjt
the bog bit the cat
gor hwu qiv pdx mlt


Gene Sequence
Intron 1
Exon A
Intron 2
aog bej qut mda mjt
the dog bit the cat
gor hwu qiv pdx mlt
Mutation in Intron
Goes unnoticed by protein
Intron 1
Exon A
Intron 2
aog bej qut mda mjt
the dog bit the cat
gdr hwu qiv pdx mlt

Lesson 3 Class Discussion of Electrophoresis and Genetic Trait

Review of DNA and Genes Answer Sheet

Review Questions

1. What is DNA?

   The instructions for building parts of the cell.

2. What does "DNA" stand for?

   DeoxyriboNucleic Acid

3. What is the four-letter DNA alphabet and what are the special rules by which the alphabet pieces bind together?

   A, C, T, and G. A binds with T, C binds with G.

4. What is a gene?

   Genes are instruction manuals for our bodies.

5. What are genes made of?

   Genes are made of DNA.

6. For what molecule do genes contain the instructions for building?

   Genes contain the instructions for building proteins.

7. What is a chromosome?

   Chromosomes are packages of compact DNA.

8. How many chromosomes does a human cell hold?

   Each human cell holds 46 chromosomes.

9. How are the human sex chromosomes labeled?

   Sex chromosomes are labeled " " and "Y".

10. What provides the "blueprint" for making a protein?

    Genes provide the blueprint for making a protein.

11. What is heredity?

    The passing of traits from parent to child.

12. Why aren't children identical to either one of their parents?

    Each parent contributes one set of chromosomes to each child. The set of chromosomes is passed on randomly, so each child receives a unique combination.

13. In humans, how many chromosomes does each parent pass on to their offspring?

    Each parent passes on 23 chromosomes to their offspring.

14. Does the second child in a family inherit the exact same chromosomes as the first? Do both babies have a complete set?

    No, the second baby's chromosomes are different from the first baby's. Yes, both babies have a complete set.

15. What is a trait?

    A trait is a notable feature or quality in a person.

16. Give an example of how an environmental factor can influence a trait.

    Answers may include one of the following: exposure to sun or hair dyes can change hair color, you can train retrievers to roll over and play dead instead of fetch, eating healthy foods and exercising can decrease the risk of heart disease.

17. Briefly explain how the Hitchhiker's Thumb trait is determined using the following words: allele, dominant, recessive, homozygous, heterozygous. You may draw pictures if you wish.

    Answers will vary. Example answer:

    If two dominant alleles are inherited, the person is homozygous and will have a hitchhikers thumb. If two recessive alleles are inherited, the person is homozygous and will have a straight thumb. If a person is heterozygous, one dominant and one recessive allele are inherited and that person will have a hitchhiker's thumb.

Lesson 3 Class Discussion of Electrophoresis and Genetic Trait

Introduction to Electrophoresis Answer Sheet

1. When you analyzed the results of your gel, did any of your experimental samples contain dyes that did not match the four reference dyes? For example, did any of your samples produce:

    a. Dyes that are a different size than any of the standard bands?

    There are three other FD&C dyes. All are negatively charged and of similar size to the four control dyes.

    b. Dyes that are a different color than any of the standard bands?

    If the candy is from outside the US, it may contain dyes other than the FD&C dyes.

    c. More than one color band?

    Many food colors are obtained by combining multiple food dyes, so some samples may produce more than a single dye band. For example, brown color is frequently a combination of Blue 1 and Red 40.

    Several dyes are too close in size and charge to separate on a 1% agarose gel, so students may see a single band for the two colors. For example, Yellow 6 and Red 40 will not separate, so students will see a single orange band if those two dyes are present in their sample.

    d. Dyes that you observed moving in the "wrong" direction (toward the cathode)?

    Although they are not common, some food dyes may be positively charged and will migrate toward the negative (cathode or) electrode.

2. We powered our electrophoresis with 9 V batteries connected in a series (multiple batteries with negative electrodes connected to positive electrodes to form a chain). For batteries connected in series, the total voltage is equal to the sum of the voltages of the individual batteries.

    a. How many batteries did we use? __**Answers will vary**___

    b. What would be the voltage if we used three batteries in series? __**27**___ volts

    c. What if we used five batteries? __**45**___ volts

    d. Would we expect our samples to migrate faster through the gel if we used three batteries or five batteries? Why?

The samples would migrate faster through the gel if five batteries are used than they would if three batteries were used. If there is more voltage, there is more current (V=IR). Current is the rate that charged molecules flow. If they can flow faster, then for the same amount of time, they will move farther.

3. For these experiments, we used a 1% agarose gel to separate the dyes. How do you think the results would differ if we used a 3% agarose gel? A 0.8% agarose gel?

   A 3% agarose gel would slow down the larger molecules more than in a 1% or 0.8% gel. Within the same time frame, all of the dyes would run farther along the 0.8% gel than in a 1% or the 3% gel.

4. What are some properties that need to be considered when picking a material to construct an electrophoresis box?

   Answers will vary but may include: The material needs to be moldable. It needs to have a melting point above 60˚C. It needs to be transparent or translucent. It needs to be able to withstand heating and cooling. It needs to be able to hold salt solutions without corroding. It needs to be non-conductive.

5. In this kit, you used zinc-coated steel paper clips to construct electrodes. Commercially-made gel boxes use platinum wire for electrodes. What are the characteristics needed for a good electrode?

   Answers will vary.

   A good electrode material must conduct electricity. All metals conduct electricity, but not all metals conduct electricity well. Poor conductors include tungsten and manganese. Good conductors include gold, silver, copper, and platinum.

   Electrode materials must be solid at working temperatures. For example, mercury conducts electricity, but it would be very difficult to make a mercury electrode for a gel box.

   Finally, the material should be as inert or non-reactive as possible. Electrodes are exposed to salt solutions and electricity. Materials that corrode easily, such as iron, make poor electrodes. Reactivity series lists of materials and their reactivity can be found on the internet. There are a number of metals that are reactive only with strongly oxidizing acids and would make good electrode materials, such as gold and platinum. Fortunately, they also meet the first two criteria.

6. Which direction would you expect a dye to run if it has no charge at pH 8?

   If it has no charge, the dye will not move out of the well and therefore will not migrate towards the anode or the cathode.

7. Calculate the rate that each dye moves in the gel.

    a. Using the photograph of your gel, measure the distance each reference dye moved from the wells in cm

    The distance each reference dye migrated in the gel will vary with the amount of time the gel was run and the number of batteries used.

    b. Divide the distance traveled by each reference dye by the time that you ran your gel (in minutes)

    The time the gel was run should be 20 minutes.

    c. Which dye has the fastest rate of movement in cm/min?

    The reference dye that has the fastest rate of movement in the gel is Yellow 5.

    d. Why might the distance travelled differ between the dyes?

    The dyes are different sizes so they will move through the agarose at different rates as the larger molecules get hung up.

Lesson 4 Introduction to Human *Alu* PV92 & Isolation of Cheek Cell DNA

Advanced Preparation

Aliquot InstaGene Matrix (15 min)

1. Thoroughly mix the InstaGene matrix by gently shaking or vortexing the bottle several times to Resuspend the matrix. Be sure that the matrix is well mixed when you aliquot it. The beads settle out of solution quickly, so gently remix the bottle several times during aliquotting.

2. Pipet 200 μl of InstaGene matrix into each screw cap tube. Distribute one tube to each student. Each student workstation should get 4 tubes of matrix for 4 students.

Prepare and aliquot saline solution (15 min)

1. Prepare a 0.9% saline solution. To a 500 ml bottle of drinking water, add 4.5 grams of noniodinated salt. Table salt is recommended. Invert the bottle until the salt goes into solution.

2. For each student, place 10 ml saline into a separate cup. Each student workstation should have 4 cups of saline for 4 students.

Set up workstations (30 min)

The kit provides enough materials for 8 student workstations. Workstations should include no more than 4 or 5 students. Carefully ensure that each workstation is equipped with the following materials.

Student workstations	**Quantity**
1.5 ml micro test tube	4
Screw cap tubes with InstaGene matrix	4
Foam micro test tube holder	2
P-20 micropipet	1
Pipet tips (filter type), 2-20 μl	4
Marking pen	1
Biological waste container	1
Cups with 10 ml 0.9% saline	4

Instructor (common) workstations	**Quantity**
P-1000 or P-200 micropipet	1
Pipet tips (filter type)	1 box
Water baths (56 °C and 100 °C)	1 each
Microcentrifuge	1
Vortexer (optional)	1

Lesson 4 Introduction to Human *Alu* PV92 & Isolation of Cheek Cell DNA

Instruction

Student workstations are set up for today's activity; cheek cell isolation. Students should come in and turn to lesson 4 in their workbooks.

20 minutes Introduction to *Alu* PV92

Review the "central dogma" they learned yesterday (DNA → pre-mRNA → mRNA → protein) with students. How does the information in DNA (genotype) get turned into a protein (phenotype)? Review the concepts of noncoding DNA and introns. Make the connection between these concepts and the *Alu* PV92 region on chromosome 16. To further explain transposable elements and the use of insertions in tracking human ancestry, show segments of the media available at (particularly "How *Alu* Jumps" and "Clip 1" from the video selection):

http://www.geneticorigins.org/pv92/aluframeset.htm

Make the (early) connection between mutations and potential genetic disorders, such as the NF-1 gene and neurofibromatosis I, as referenced in the lesson.

Move to introducing the investigation the students will undergo. Explain they will be using polymerase chain amplification (PCR) and agarose gel electrophoresis to determine their own genotype for *Alu* PV92. PCR is simply making copies selectively in order to isolate the gene of interest. Review with the class how the genotype can be determined by looking at the different sized fragments separated on a gel. Ask students to draw gels and predict what they would see for each type of genotype (+/+, +/-, -/-). Which of these is a homozygous genotype? Where would the bands be located? How would they look in comparison to a standard?

30 minutes Activity

Review the tasks involved in PCR and agarose gel electrophoresis as is presented at the end of "What Is *Alu* PV92?". Today the students will complete the first task; isolating DNA from their cheek cells. Review the reagents and equipment required for this protocol.

The most important goal for the lab is for the student to get a viable sample. Help students work through issues with spinning down their sample and removing the supernatant. To spur discussion and critical thinking, the instructor should ask students questions regarding the purpose of each step of the procedure as the group moves through them.

10 minutes Wrap-Up

Samples should be kept on ice. As a review, ask students the following "exit" questions:

1. What is needed from the cells for the next steps of agarose gel electrophoresis?

   The genomic DNA released from the nuclei of the cells is needed for the PCR reaction.

2. What structures must be broken to release the DNA from cells?

   The cell and nuclear membranes must be broken to release the template DNA into the solution.

3. If we just want a segment of the DNA to test (the part that may or may not contain Alu PV92) what do we have to do to the DNA before we can analyze it?

   Isolate that area (through amplification).

Explain to the students that in the next lesson they will follow protocol for 'amplifying" their DNA. Remind them that the cells they have isolated today contain their entire DNA profile, and they are interested in only a small portion of that sample.

Lesson 5 PCR Amplification of DNA

Advanced Preparation

Prepare complete master mix and aliquot (15 min)

Prepare no more than 30 minutes before PCR cycling

1. Pipet 1,100 μl of master mix into a labeled micro test tube. If you choose to amplify 16 student samples or less, divide the master mix into two tubes with 550 μl each. One tube will be used immediately, and the remaining master mix can be refrozen for later use.

2. For 32 students or 8 student workstations (halve for 16 students), label 8 micro test tubes "Master" and place the tubes on ice.

3. Add 22 μl of the primer mix to the 1,100 μl master mix or add 11 μl of primer mix to the 550 μl master mix. Vortex 10 seconds to mix. It is imperative that the master mix be evenly mixed after the addition of the primers. The solution should be yellow.

   The primers are supplied as a concentrated yellow solution in a Tris buffer. Since the primers are much more stable in a concentrated form, add the primers to the master mix just prior to the beginning of the laboratory exercise – **not more than 15-30 minutes** before the PCR amplification.

4. Aliquot 95 μl of the complete master mix into the 8 micro test tubes labeled "Master", supplying one tube for each student workstation (1-8). Save the remaining complete master mix for the positive control reactions. Place these tubes on ice until they will be used.

Set up control PCR reactions (20 min)

1. Label the control PCR tubes: +/+, -/-, and +/-. If you will be using the entire kit with a single lab period set up 4 of each control or 12 tubes total. If you will be splitting the kit between two lab periods, set up 2 of each control, or 6 tube total. The unused control solutions should be stored in the freezer until used.

   a. Pipet 20 μl of the +/+ template into each +/+ PCR tube.

   b. Pipet 20 μl of the -/- template into each -/- PCR tube.

   c. Pipet 20 μl of the +/- template into each +/- PCR tube.

2. Pipet 20 μl of the complete master mix into each of the control tubes. **Use a fresh tip for each tube**.

3. Place the tubes on ice until ready to load into the thermal cycler. Amplify PCR control samples along with student samples during this lesson.

Program thermal cycler and set up workstations (30 min)

The thermal cycler should be programmed for 3 steps in cycle 2, which will repeat 40 times. The final cycle 3 ensures that the final extension reaction goes to completion and all possible PCR products are made. The PCR reaction will take approximately 3.5 hours.

Cycle	Step	Function	Temperature	Time
1	Step 1	Pre-denaturation	94°C	2 minutes
	Repeat 1 time			
2	Step 1	Denature	94°C	1 minute
	Step 2	Anneal	60°C	1 minute
	Step 3	Extend	72°C	2 minutes
	Repeat 40 times			
3	Step 1	Final Extension	72°C	10 minutes
	Repeat 1 time			
*	Step 1	Hold	4°C	Infinity

The kit provides enough materials for 8 student workstations. Workstations should include no more than 4 or 5 students. Carefully ensure that each workstation is equipped with the following materials.

Student workstations	Quantity
PCR tubes	4
Micro test tubes, capless	4
Complete master mix (containing primers) on ice	1 tube
P-20 micropipet	1
Pipet tips (filter type), 2-20 μl	8
Foam micro test tube holder	2
Ice bucket with ice	1
Marking pen	1
Biological waste container	1

Instructor (common) workstations	Quantity
Thermal cycler	1
Microcentrifuge	1

Lesson 5 PCR Amplification of DNA

Instruction

Student workstations are set up for today's activity; polymerase chain reaction. Students should come in and turn to lesson 5 in their workbooks. To prepare, pull up the websites cited in the "Introduction to PCR" lesson.

10 minutes Introduction

The goal of the last lab was to get isolate your DNA sample. However, explain to students, this is a sample containing your total DNA – all of the DNA that makes up your entire genome. We are interested in analyzing just a very small segment of this total DNA – the *Alu* PV92 region. The goal of today's lab is to amplify this specific region of DNA – or, make multiple copies of it. Ask students if they might know what is needed to accomplish this task. To round out understanding, further explain that the master mix that will be added to student samples, along with the primers, for the PCR is a mixture of dNTPs (dATP, dTTP, dCTP, and dGTP), buffer, and *Taq* DNA polymerase. *Taq* DNA polymerase is the enzyme that builds new DNA strands, the dNTPs are the building blocks, the primers provide the starting template for these copies, and the buffer provides the medium in which the reaction takes place. This will result in a sample where there are vastly more copies of this specific region compared to all other DNA.

15 minutes Activity

The first task is to be sure each student understand the needed supplies; PCR tube, capless tube, micro test tube holder. It is **very important** that student clearly label their PCR tubes, either with their initials or some symbol they can use for identification. Once the student has the needed supplies, be sure they understand the materials or reagents they are using; their personal sample and the master mix for the PCR reaction.

Once the student has all needed materials and supplies arranged, the student will need to use pipetting skills to transfer the personal sample from its test tube into the PCR micro tube. **It is very important that the student draw this sample from the supernatant and not disturb the matrix bead at the bottom of the sample**.

Using a new pipet tip, the student will then transfer master mix into the PCR tube. This will need to be mixed lightly with the sample. Once this is done, the students can put their PCR tubes into a holder if a thermal cycler is not immediately available. Store the samples on ice until you have all that will need to be run. Review the table which shows the programming for the thermal cycler. This will be discussed further in the next section of this lesson.

25 minutes Introduce polymerase chain reaction

Help students make the connection between isolating DNA and then making enough copies of the region of interest to further investigate. Review the following videos:

http://www.dnalc.org/resources/animations/pcr.html

http://learn.genetics.utah.edu/content/labs/pcr/

http://www.carolina.com/teacher-resources/Video/dnalc-cycles-polymerase-chain-reaction-pcr-3d-animation/tr25106.tr?s_cid=em_ctGen_201403&utm_source=bronto&utm_medium=email&utm_term=Image+-+http%3A%2F%2Fwww.carolina.com%2Fteacher-resources%2FVideo%2Fdnalc-cycles-polymerase-chain-reaction-pcr-3d-animation%2Ftr25106.tr%3Fs_cid%3Dem_ctGen_201403&utm_content=herringd%40gvsu.edu&utm_campaign=2014+March+General+Tips

During the videos, point out concepts such as:

1. Complementary DNA strand hybridization

   The primers provided in this experiment will flank the segment of interest, where the DNA polymerase will begin synthesizing copies. Complementary strand hybridization takes place when two different primers bind to each of their respective complementary base sequences on the template DNA.

2. DNA strand synthesis via DNA polymerase.

   Taq DNA polymerase extends the annealed primers by "reading" the template strand and synthesizing the complementary sequence, replicating the two template DNA strands. This polymerase was isolated from a heat-stable bacterium (*Thermus aquaticus*) which in nature lives within high temperature stream. For this reason, these enzymes have evolved to withstand high temperature (94 °C) and can be used in the PCR process.

During the videos, discuss what is occurring at each step of the reaction:

- In denaturation, the reaction mixture is heated to 94 °C for 1 minute, which results in the melting or separation of the double-stranded DNA template into two single stranded molecules. In PCR amplification, the DNA templates must be separated before the polymerase can generate a new copy. The high temperature required to melt the DNA strands normally would destroy most active enzymes, but *Taq* polymerase is stable and active at high temperatures.

- During the annealing step, the oligonucleotide primers "anneal to" or find their complementary sequences on the two single-stranded template strands of DNA. In these annealed positions, they act as primers for *Taq* DNA polymerase. They are called primers because they "prime" the synthesis of a new strand by providing a short sequence of double-stranded DNA for *Taq* polymerase to extend from and build a new complementary strand. Binding of the primers to their template sequences is also highly dependent on temperature. In this exercise, a 60 °C annealing temperature is optimum for primer binding.

- During the extension step, the job of *Taq* polymerase is to add nucleotides (A, T, G, and C) one at a time to the primer to create a complementary copy of the DNA template. During polymerization the reaction temperature is 72 °C, the temperature that produces optimal *Taq* polymerase activity. The three steps of denaturation, annealing, and extension form one "cycle" of PCR. A compete PCR amplification undergoes 40 cycles.

- The entire 40 cycle reaction is carried out in a test tube that has been placed into a thermal cycler. The thermal cycler contains an aluminum block that holds the samples and can be rapidly heated and cooled across broad temperature differences. The rapid heating and cooling of this thermal block is known as temperature cycling or thermal cycling.

10 minutes Wrap-Up

Inform the students that the PCR will run after class and their amplified samples will be ready for them next class period. Once the samples are done running, store them at 4°C until they are needed again.

If students did not complete the *purpose of each reaction mixture part* of Understanding PCR, they should complete this as homework. Remind students that in the next class period they will be running their amplified samples on an agarose gel.

Lesson 5 PCR Amplification of DNA

Understanding PCR

Do you know the purpose of each component in the reaction mixture? Fill in your answers below:

1. DNA template – **containing the intact DNA sequence to be amplified**.
2. Individual deoxynucleotides – **A, T, G, and C; raw material of DNA**.
3. DNA polymerase – **an enzyme that assembles the nucleotides into a new DNA chain**.
4. Magnesium ions – **a cofactor (catalyst) required by DNA polymerase**.
5. Oligonucleotide primers – **pieces of DNA complementary to the template that tell the DNA polymerase exactly where to attach and start making copies**.
6. Salt buffer – **provides the optimum ionic environment and pH; a medium in which the polymerase chain reaction occurs**.

Lesson 6 DNA Analysis by Gel Electrophoresis

Advanced Preparation

Prepare TAE buffer and molten agarose (20 min)

Prepare electrophoresis buffer

The electrophoresis buffer is provided as a 50x concentrated solution. 1x TAE buffer is needed to make the agarose gel and is also required for each electrophoresis chamber. Three liters of 1x TAE buffer will be sufficient to run 8 electrophoresis chambers and pour 8 agarose gels. To make 3 L of 1x TAE from a 50x TAE concentrate, add 60 ml of concentrate to 2.94 L of distilled water. Aliquot 250 ml of buffer into smaller Erlenmeyer flasks for student use.

Make agarose solution

i) The recommended gel concentration for this application is 1% agarose. This agarose concentration provides excellent resolution and minimizes run time required for electrophoretic separation of PCR fragments. To make a 1% solution, add 1 g of agarose to 100 ml of 1x TAE electrophoresis buffer. For 8 gels, you will need approximately 350 ml of molten agarose (3.5 g agarose per 350 ml of 1x TAE buffer). The agarose must be made using electrophoresis buffer, not water.

ii) Add the agarose powder to a suitable container (for example, use a 500 ml Erlenmeyer flask for 200 ml or less). Add the appropriate amount of 1x TAE electrophoresis buffer. If clumps of agarose are visible, swirl the flask to mix.

iii) While heating the solution, some of the volume will be lost to evaporation. Prior to heating, mark the volume level on the flask or weigh the flask and content and record the weight.

iv) Dissolve the agarose by heating in a microwave oven. If a microwave is not available, the solution can be heated on a magnetic hot plate.

> **Caution**: Always wear protective gloves, goggles, and lab coat while preparing and casting gels. Molten agarose or the flasks containing hot agarose can cause severe burns if allowed to contact skin.

a. Microwave oven method

This technique is the fastest and safest way to dissolve agarose. Place the gel solution in an appropriate bottle or flask into the microwave. If you are using a bottle, **be sure to loosen the cap before heating**. The ideal microwave setting will depend on the volume of agarose solution that you are preparing and on the power of the microwave oven. For small volumes, microwave solution for 1 min, swirl the solution, then microwave in 20-30 sec intervals,

swirling after each, until **all** of the small transparent agarose particles are dissolved. For larger volumes (e.g. 400 ml), heat initially for 2-3 min, swirl the solution, then microwave in 30-45 sec intervals, swirling after each, until **all** of the small transparent agarose particles are dissolved.

Add water to the agarose solution to bring it back to the original volume and swirl to mix completely. Cool agarose solution to 55-60 °C before pouring gels. If students are going to pour their own gels, keep the agarose at 55-60 °C until it is ready to be poured by either stirring on a hot plate or in a water bath. If the agarose is kept warm in a water bath, make sure to swirl it thoroughly to mix the solution immediately before the gels are poured.

b. Magnetic hot plate method

Add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Boil the solution until **all** of the small transparent agarose particles are dissolved.

Add water to the agarose solution to bring it to the original volume and swirl to mix completely. Cool agarose gels to 55-60 °C before pouring gels. If students are going to pour their own gels, keep the agarose at 55-60 °C until it is ready to be poured by stirring on a hotplate. Aliquot 50 ml of molten agarose into smaller Erlenmeyer flasks for student use.

<u>Prepare reagents (20 min)</u>

Prepare positive control samples

Add 10 µl of PV92 XC loading dye into each amplified positive control sample (+/+, -/-, +/-). Place the tubes at the teacher's workstation. Either you or a student group will load the positive and negative control samples on each gel.

Aliquot DNA size standards

Aliquot 11 µl of the EZ Load molecular mass ruler into 8 microtubes and label "MMR". The sizes of the DNA standards bands are 1,000 bp, 700 bp, 500 bp, 200 bp, and 100 bp.

Aliquot PV92 XC loading dye

Label 8 screw cap tubes "LD" for loading dye, and aliquot 50 µl into each tube. Distribute to student workstations.

Prepare Fast Blast DNA stain (15 min)

Fast Blast DNA stain is provided as a 500x concentrate that must be diluted prior to use. The stain can be used as a quick stain when diluted to 100x to allow the visualization of DNA within 12-15 minutes, or it can be used as an overnight stain when diluted to 1x. When an agarose gel is immersed in Fast Blast DNA stain, the dye molecules attach to the DNA molecules trapped in the agarose gel. When the DNA bands are visible, your student can determine their genotypes for the *Alu* insert.

Fast Blast DNA stain is a convenient, safe, and nontoxic alternative to ethidium bromide for the detection of DNA in agarose gels following electrophoresis. Fast Blast contains a cationic compound that belongs to the thiazin family of dyes. The positively charged dye molecules are attracted to and bind to the negatively charged phosphate groups on DNA. The proprietary dye formula stains DNA deep blue in agarose gels and provides vivid, consistent results.

Warning: Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes. Dispose of the staining solutions according to protocols at your facility. Use either 10% bleach solution or 70% alcohol solution to remove Fast Blast from most surfaces.

1. To prepare 100x stain (for quick staining), dilute 100 ml of 500x Fast Blast with 400 ml of distilled or deionized water in an appropriately sized flask or bottle and mix. Cover the flask and store at room temperature until ready to use.

2. To prepare 1x stain (for overnight staining), dilute 1 ml of 500x Fast Blast with 499 ml of distilled or deionized water in an appropriately sized flask or bottle and mix. Cover the flask and store at room temperature until ready to use.

Notes:

- We recommend using 120 ml of diluted Fast Blast to stain two 7 x 7 cm or 7 x 10 cm agarose gels in individual staining trays provided in the kit (you may want to notch the gel corners for identification). If alternative staining trays are used, add a sufficient volume of staining solution to completely submerge the gels.
- Following electrophoresis, agarose gels must be removed from their gel trays before being placed in the staining solution. This is easily accomplished by holding the base of the gel tray in one hand and gently pushing the gel out with the thumb of the other hand.
- Because the gel is fragile, special attention must be given when handling it. We highly recommend using a large spatula or other supportive surface to transfer the gel from one container to another during the destaining steps involved in the quick staining protocol.
- Destaining (when performing the quick staining protocol) requires the use of at least one large-volume container, capable of holding at least 500 ml, at each student workstation. Each student

team may utilize separate washing containers for each wash step, or simply use a single container that is emptied after each wash and refilled for the next wash.

- It is crucial that you shake gels gently and intermittently during the overnight staining in Fast Blast DNA stain, small DNA fragments tend to diffuse without shaking.
- 100x Fast Blast can be reused at least 7 times.
- No washing or destaining is required when using the overnight staining protocol.

Set up workstations (30 min)

The kit provides enough materials for 8 student workstations. Workstations should include no more than 4 or 5 students. Carefully ensure that each workstation is equipped with the following materials.

Student workstations	**Quantity**
Molten agarose	50 ml
PCR samples	1 per student
PV92 XC DNA loading dye	1 tube
P-20 micropipet	1
Pipet tips (filter type), 2-20 μl	12
EZ Load molecular mass ruler (DNA standards)	1 tube
Marking pen	1
Foam micro test tube holder	2
Gel box and power supply	1
Gel staining tray	1 per 2 stations
Fast Blast DNA stain, 1x	120 ml per 2 stations
Gel support film (optional)	1
Clear acetate sheets for tracing gels (optional)	1
Warm tap water for destaining	1.5-2 L per 2 stations
Large containers for destaining	1-3 per 2 stations
Biological waste container	1

Instructor (common) workstations	**Quantity**
1x TAE electrophoresis buffer	275 ml per gel box
Amplified positive control samples (4 each)	12
PV92 homozygous (+/+)	
PV92 homozygous (-/-)	
PV92 heterozygous (+/-)	
Rocking platform (optional)	1
Microcentrifuge	1

Lesson 6 DNA Analysis by Gel Electrophoresis

Instruction

10 minutes Instructions

Explain to the students that they will actually be running an actual agarose gel electrophoresis today using their personal DNA samples. It will be their job to determine the genotype of their personal DNA sample by comparing it to the standard provided. Review the various reagents and equipment, making reference to the items they explored in the previous lessons.

40 minutes Activity

The first task is to pour the agarose. Students may have to use the gel chamber in one way as a mold for their gels, and once the gel has solidified, reconstruct the gel chamber to set it up for electrophoresis. Students must be careful with the fragile gel as they set up the electrophoresis apparatus and when they pull the comb out which formed the wells.

As they prepare their samples, be sure students spin down their samples to bring all the liquid to the bottom of their microcentrifuge tubes. Be sure they are using a different pipet tip as they add loading dye to each sample since each sample contains DNA from different students.

Once samples are loaded, assist students as they hook up their power supply. Ask the students which electrode, (+) or (-), should be at each end of the gel chamber. The students should watch to be sure their samples start moving through the gel and in the correct direction.

If there is enough class time, students can monitor their gels until they finish running and stain their gels in order to view the results. After the staining process, students will be able to view their results with the naked eye and photograph their gels. If classroom time is too short to have students finish running their gels, the instructor will need to monitor them. Once they are done running, the instructor should also proceed with the staining/destaining and photography of results.

10 minutes Wrap-Up

If time permits, while students are waiting for their gels to run, they can begin working on the discussion questions for this lesson. These can be completed as homework. Inform students that they will analyze their results in the next class period to determine what genotype they are at the *Alu* PV92 locus.

Lesson 6 DNA Analysis by Gel Electrophoresis

Discussion Questions Answer Sheet

1. Explain the differences between an intron and an exon.

   Introns, or intervening sequences, do not code for protein sequences and are spliced out of mRNA molecules before the mRNA leaves the nucleus. Exons code for the protein sequence and remain in the mRNA, are transported out of the nucleus, where they are finally translated into protein on ribosomes.

2. Explain how agarose electrophoresis separates DNA fragments. Why does a smaller DNA fragment move faster than a larger one?

   PCR fragments are separated in an electrophoretic field because DNA is a negatively charged molecule which moves when an electric field is applied to it. Since DNA is negatively charged it migrates toward the positive (red) electrode.

   The agarose acts as a sieve to separate the charged DNA molecules according to size. Large molecules of DNA move slowly through the agarose, while smaller molecules of DNA are less obstructed and move faster through the matrix of agarose.

3. What kinds of controls are run in this experiment? Why are they important? Could others be used?

   The controls that are run in this experiment are the homozygous +/+, homozygous -/-, and heterozygous +/- known samples. These bands have known base-pair lengths and can be used in comparison to unknown student samples.

Lesson 7 Analysis and Interpretation of Your Results

Instruction

10 minutes Instructions

Review with students that over the past few days they have isolated DNA, amplified a segment of interest, and performed an agarose gel electrophoresis. Review the goal of this lab procedure; to identify each student's genotype at the *Alu* PV92 locus on chromosome 16, and ask students to review their predictions. Today, they will review their results to determine their genotype and compare it to others in the class.

40 minutes Activity

Whether the students were able to see their gels run to completion or the instructor had to finish running them and photograph them, allow students time with their results. Ask them what they notice about their gels. Can they see all the bands easily? Are some they all dark or light? Why would some be darker than others? Did their controls work? What results would they expect from each control? Are they able to identify their standard and label each band with the appropriate size according to the key?

The students may need to review the introductory information regarding Alu, but after this review, they should be able to work through the example in question 1 and label the genotype of each lane. Once they have practiced on the example gel in their workbook, they should then try to determine the genotype of each sample on their actual gel.

Once students have identified their genotypes, they should move on to question 2 to determine the observed genotype and allele frequencies of the class. Students should think about the relationship between genotype and alleles to determine a table appropriate to display the class results for each along with the frequencies of each. Encourage discussion at this point to assist students in arriving at an answer. For example, each person has two alleles at the Alu PV92 locus. What form can each allele take (+ or -)? How many combinations are possible resulting in a genotype (-/-, -/+, and +/+)? How can the student display the data for each genotype and allele type?

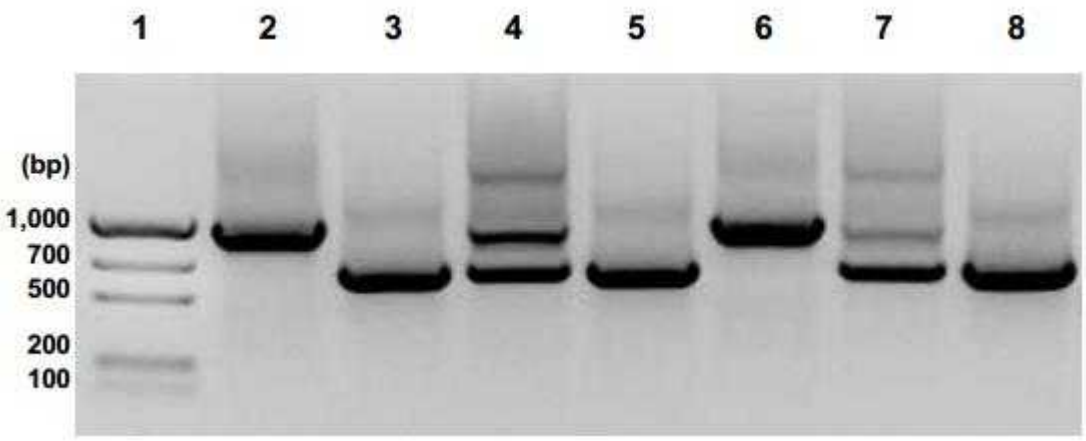
10 minutes Wrap-Up

Remind students that Alu PV92 is located in a noncoding region of their DNA. However, ask them to think about the implications results like these might have for genes that are in coding regions. Inform them that in the next portion of the unit, they will begin looking at genes which have the ability to cause disease. How might techniques like agarose gel electrophoresis be useful in this area of medicine?

Lesson 7 Analysis and Interpretation of Your Results

Interpreting Your Gel Example Answer Sheet

1. Determine your PV92 genotype. Practice with the gel below. Based on what you know about the *Alu* locus on chromosome 16, can you determine what genotype each lane displays? How do you know? Label each lane on the gel below and then use it as a key in determining the genotypes represented on your own gel photograph.



Other notes to keep in mind when analyzing your gel:

a. It is common to see a diffuse (fuzzy) band that runs ahead of the 121-bp marker. This is "primer dimer," an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. The presence of primer dimer, in the absence of other bands, confirms that the reaction contained all components necessary for amplification.

b. Additional faint bands at other positions occur when the primers bind to chromosomal loci other than the PV92 locus and give rise to "nonspecific" amplification products.

My sample shows band(s) at: _____**example: 941 bp and 641 bp**_____
My *Alu* PV92 genotype is: _____**+/-**_____

2. Why do the two possible PCR products differ in size by 300 base pairs?

   The PCR primers amplify a 641 bp fragment within the PV92 region. Certain individuals contain a 300 bp Alu repeat within this region of chromosome 16, and amplification from these individuals produces a 941 bp fragment. Thus, the 300 bp difference in size is due to an insertion of a 300 bp Alu repeat.

3. Determine the observed genotype and allele frequencies for your class using the equations below. Determine what table best represents your data, draw it in the space below, and record your answers there.

$$\text{genotype frequency (\%)} = \frac{\text{number of students of X genotype}}{\text{total student samples}} \times 100$$

$$\text{allele frequency (\%)} = \frac{\text{number of X alleles}}{\text{total alleles in sample}} \times 100$$

For additional student support:

a. Count the number of students of each genotype: +/+, +/–, and –/–. Exclude from the analysis any students whose genotypes could not be determined.

b. First, multiply the number of students of each genotype by the number of + or – alleles in that genotype. Remember that each +/+ or –/– student contributes 2 copies of that allele, while each +/– student contributes one of each allele. Then add up the total number of copies of each allele. The TOTAL number of alleles in the sample is twice the number of students.

Example Table (answers will vary depending on actual class results):

Genotype Frequency	# Students	Genotype	+ Allele (#)	– Allele (#)
		+/+		
		+/–		
		–/–		
TOTALS>				
		Allele Frequency>		

Lesson 8 Molecular Techniques and Genetic Diseases/Disorders

Instruction

10 minutes Instructions

Explain to students that they have now learned the technique of agarose gel electrophoresis, but this technique is only as useful as its application. Drawing again from the introductory material in the first three lesson of this unit, and rounding out the big picture, students will explore gene inheritance and mutation to understand the implications these processes have on disease. In this lesson, they will explore how molecular techniques, like agarose gel electrophoresis, can help inform the medical community and how this information can be used to better detect and treat genetic disorders and diseases.

40 minutes Activity

This lesson's class will allow free exploration of the topic of molecular techniques and genetic disease. Students should spend time exploring the information at the links provided. The instructor should allow some free time for this, but move about the classroom to be sure students remain on task. Some of the videos are quite long. Suggest students pay particular attention to segments such as:

Scanning Life's Matrix: Genes, Proteins, and Small Molecules (http://www.hhmi.org/biointeractive/human-genetics-new-guide-medicine)

Lecture 1 – Reading Genes and Genomes

9. Reading DNA helps us understand disease

11. Genetic basis of cystic fibrosis

17. Q&A: Does "junk" DNA cause problems in interpreting the genome?

18. Q&A: Are most diseases caused by small changes in DNA?

20. Q&A: Could you talk about gene therapy?

Lecture 3 – Human Genomics: A New Guide for Medicine

10. Single nucleotide polymorphism (SNP) can affect Alzheimer disease

11. Other examples of variations affecting diseases

21. Can differences in leukemia be detected by microscopy?

25. Using microarrays to detect the activities of all the genes in a tumor

Medicine in the Genomic Era
(http://www.hhmi.org/biointeractive/medicine-genomic-era)

Lecture 1 – Sizing up the Brain Gene by Gene

16. Mutations in *AKT3* gene correlate with brain size disorders

27. Using genetic markers to find microcephaly genes

37. Q&A: Can microcephaly result from a somatic mutation?

Lecture 2 – Cancer as a Genetic Disease

10. Mutated proto-oncogenes can become oncogenes and cause cancer

16. Q&A: How are cancer cells detected?

17. Q&A: How common are point mutations in different cancer genes?

27. Molecular technologies catalyzed targeted therapies development

Lecture 3 – Decoding the Autism Puzzle

9. Genetic disorders may be recessive, dominant, or multigenic

10. Not all genetic traits are inherited

Lecture 4 – From Cancer Genomics to Cancer Drugs

4. Cancer mutations include substitutions, INDELS, and translocations

14. Identifying cancer genes gives insights into treatment strategies

21. Presence of multiple mutations affects how well a drug works

Of Hearts and Hypertension: Blazing Genetic Trails
(http://www.hhmi.org/biointeractive/telltale-genes-charting-human-disease)

Lecture 2 – Telltale Genes: Charting Human Disease

7. Using DNA markers to map disease genes

Lecture 3 – Heartbreak: Of Mutations and Maladies

5. Using genetics to diagnose cardiomyopathy

6. Detecting the disease when no symptoms are present

After about 20 minutes of exploration, the instructor should initiate individual or small group discussion if they are not already occurring. Encourage students to think about the information they are exploring in a way that begins to connect these various topics. What other techniques seem interesting to you? How do they work at the molecular level? What uses did the resources cite for these techniques? Can you name one disease in which these techniques have been used, either for diagnostic or treatment purposes? For that disease, what type of molecular abnormality did it find? Do these abnormalities typically occur through inheritance or mutation? If it is a mutation, what occurred at the genetic level to cause the mutation? What was the result of the mutation event? How does this new genetic information get translated? What problem does it cause at the protein level, the tissue level, the organ level? Does the treatment for any of these diseases differ based on a patient's genetic profile? Why, what impact does the genetic variation have on treatment options?

10 minutes Wrap-Up

Encourage students, if they can, to continue exploring this information at home. They should also complete the reflection portion of the worksheet. This will enable them to move on to the next lesson where they investigate how the genetic code can be used to treat diseases more specifically.

Lesson 8 Molecular Techniques and Genetic Diseases/Disorders

Reflection Example Answer Sheet

1. What are some examples of how gel electrophoresis can help detect a genetic disorder or disease?

   Answers will vary: diagnosing a particular allele variant that is known to cause a particular disease, diagnosing a mutation that has changed the length of the gene region, etc.

2. What is another technique for detecting genetic disorders or diseases that was new to you? Please describe how the process works below.

   Answers will vary: protein electrophoresis, microarray, in situ hybridization, etc.

3. In addition to determining genetic disorders, what might be other uses for running a gel electrophoresis?

   Answers will vary: Forensic investigation, genetic counselors during pregnancy counseling, paternity testing, human descent/evolution studies, tracking of epidemics/pandemics, etc.

Lesson 9 Pharmacogenomics

Instruction

10 minutes Instructions

Today's lesson continues the investigation of molecular techniques and their application to medical treatments. Today's exploration focuses on how this information can be used to improve the efficacy of medical treatments and avoid unintended consequences.

40 minutes Activity

This lesson's class will allow free exploration of the how pharmacogenetics can lead to more specialized treatment of disease. Students should spend time exploring the information at the links provided. The instructor should allow some free time for this, but move about the classroom to be sure students remain on task. Engage students in conversation by asking them questions such as; are they familiar with any medical treatments that target specific genotypes? What diseases do they think would benefit the most from this sort of targeting? Is this an area of medicine they might be interested in going into? What professions do they think are available to bring such medical treatments to market?

10 minutes Wrap-Up

Encourage students, if they can, to continue exploring this information at home. They should also complete the reflection portion of the worksheet. This will provide them with a broader understanding of how the genetic code can be used to consider how best to approach the treatment of disease medically. The next lesson will ask the students to investigate a variety of genetic diseases in more detail.

Lesson 9 Pharmacogenomics

Reflection Example Answer Sheet

1. Give an example of how molecular techniques help personalize medical treatments. What are the benefits for tailoring medical treatments to a person's genotype for this particular disease? Are there any negative consequences?

   Answers will vary: positive - more efficacious medical treatments, quicker treatment/recovery times, avoid medical side effects; negative increased cost, policy issues, etc.

2. In addition to designing medical treatments, what other reasons are there for knowing a person's genotype or genome?

   Answers will vary: disease screening, understanding family history, avoiding confounding medical variables, etc.

3. What do you think about the challenges and issues in personalized medicine and pharmacogenomics?

   Answers will vary: good for small group discussion.

Lesson 10 Introduction to Genetic Based Diseases/Disorders

Instruction

10 minutes Instructions

Now that students have a basic understanding of genetics and molecular techniques, in this lesson, they will begin to explore various genetic disorders. By the end of the lesson, the students should have a few genetic disorders in mind that they would like to explore in further detail.

40 minutes Activity

This lesson's class will allow free exploration of genetic diseases and disorders. Students should spend time exploring the information at the links provided. The instructor should allow some free time for this, but move about the classroom to be sure students remain on task. Engage students in discussion by asking questions such as; which diseases seem interesting for further study? What is it about these diseases that intrigue you? Are you finding that many of the diseases stem from the same genetic basis or are the genetic cause varied? What other medical characteristics do the patients with these diseases share? Do any of these diseases already have a pharmacogenomic treatment?

10 minutes Wrap-Up

Encourage students, if they can, to continue exploring this information at home. They should also complete the reflection portion of the worksheet. This will enable them to move on to the next lesson where they investigate a particular genetic disease in more detail with a group in order to prepare an information presentation for the class.

Lesson 10 Introduction to Genetic Based Diseases/Disorders

Reflection Example Answer Sheet

1. Which genetic disorder or disease is of interest to you; which might you be interested in exploring further? Please describe it below.

   Answers will vary, but you should encourage students to start exploring the aspects of the disease they will need to report on in the next lesson:

   - **What is the disease?**
   - **How do people get the disease?**
   - **What are the symptoms of the disease?**
   - **How do doctors diagnose the disease?**
   - **How is the disease treated?**
   - **Interesting facts about the disease?**

2. Was there a disorder or disease described here that you previously did not know had a genetic basis? What else was new or surprising to you?

   Answers will vary: good for small group discussion.

3. What are the different types of genetic problems that might lead to the formation of a genetic disorder or disease? Do you understand the molecular mechanism by which they occur?

   Answers will vary: good for small group discussion; various mutation mechanisms, loss or addition of chromosome, translocation, etc.

Lesson 11 Genetic Disorder/Disease Presentations

Instruction

20 minutes Instructions

Ask students to pick one of the genetic disorders they explored in the last lesson for a presentation where they will provide more specific information on this disease to their classmates. They may form groups of 2 to 4. Review the requirements for the presentation, emphasizing the information they should provide. Make connections to the concepts they have covered in the unit up to this point.

How do people get the disease? Lessons 1, 2, and 3

What is the disease? Lessons 1, 2, and 3

What are the symptoms of the disease? Lesson 10

How do doctors diagnose the disease? Lessons 3, 4, 5, 6, 7, 8

How is the disease treated? Lessons 8, 9, 10

Interesting facts about the disease? This is just an "extra" or "fun" category where students can include pieces of information which will make the presentation more applicable or memorable.

1-3 days Group Work

Allow the students in-class time to work with their groups, where they have the access to resources they may need. Monitor the class to be sure students are staying on task. To assist in this, ask them different variations on the questions above to check their understanding and progress.

1-2 days Presentations

Allot 10-15 minutes for each presentation. Student presentations should be followed by questions from both the teacher and other classmates (asking questions of other groups could be considered part of the grade on the presentation). These questions should lead to short class discussions regarding the genetic basis of the disease, what impact this has on the function of the gene which causes the disease, and what could be done to correct it. Feedback should be given within a few days of the presentation and address students' ability to address the required subject knowledge as well as their general presentation skills.

Lesson 11 Genetic Disorder/Disease Presentations

Presentation Grading Rubric

	Exceeds Standard	Meets Standard	Emerging	Attempt Made
Subject Knowledge	Demonstrates mastery of the topic in each of the following categories; what the disease is, how people get it, symptoms, diagnosis, treatment, and interesting facts.	Demonstrates accurate knowledge of the topic in each of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment	Demonstrates only some knowledge of the topic, and is missing one or two of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment	Demonstrates little knowledge of the topic, and is missing more than three of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment
Organization and Coherence	Organizes information coherently, stays on the topic	Organizes most information, stays on the topic	Generally organizes information, occasionally strays from the topic	Poorly organizes information, often strays from the topic
Physical Presentation	Always speaks clearly/loudly, actively engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Usually speaks clearly/loudly, usually engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Speaks clearly/loudly, occasionally engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Does not speak clearly/loudly, neglects to engage the audience, rarely makes and maintains eye contact or uses movement to focus attention/interest
Language Convention	Uses appropriate grammar and vocabulary	Mostly uses appropriate grammar and vocabulary	Makes some errors in grammar and vocabulary	Makes many mistakes in grammar and vocabulary
Visual Aids	Creatively uses a variety of effective visual aids and/or other methods of delivery	Uses visual aids moderately effectively and/or other methods of delivery	Moderately ineffective use of some visual aids and/or other methods of delivery	Does not use of visual aids and/or other methods of delivery
Scholarly Sources	More than two additional sources included and appropriately cited	Two additional scholarly sources included and appropriately cited	Either only one additional scholarly source included or not appropriately cited	Lacking two additional scholarly sources and not appropriately cited

Lesson 11 Genetic Disorder/Disease Presentations

Presentation Evaluation Form

(Reference: Rubric for Oral Presentations, New England Association of School and Colleges, Commission on Public Secondary Schools)

Group Members: _____

_____

_____

Topic:_____

	Exceeds Standard	Meets Standard	Emerging	Attempt Made	Total
Subject Knowledge	60	50	40	30	
Organization & Coherence	5	4	3	2	
Physical Presentation	10	8	6	4	
Language Convention	5	4	3	2	
Visual Aids	10	8	6	4	
Scholarly Sources	10	8	6	4	
				Total	**100**

Comments:_____

_____

_____

_____

Student's Manual

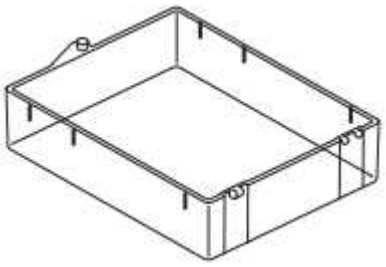
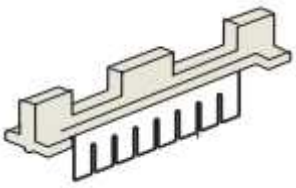
Lesson 1 Electrophoresis & Genetic Trait Exploration

Identifying the Components

Agarose gel electrophoresis is a method that uses electrical current to separate macromolecules, such as DNA, RNA, and proteins, and their fragments based on their size and charge for further analysis. Before you can use this technique, though, you need to know what materials you'll be working with during the lab. Take a look at the components, both materials and equipment, in front of you on your lab bench and at the shared table for the lab. Is there anything you recognize right away? What are some things you have never seen before? Can you work with your lab mates to identify the different components and take a guess at what they might be used for during agarose gel electrophoresis? Below are some helpful pictures and names for the different components you might encounter. See if you can match any of them with one another.

Word Bank		
Wells	**Solidified Agarose Gel**	**Leads**
Buffer	**Pipet Tip**	**Comb**
Microcentrifuge Tubes	**Molten Agarose Gel**	**Centrifuge**
Electrodes	**Samples (Known and Unknown)**	**Pipet**
Power Supply		**Gel Electrophoresis Chamber**

Select words from the word bank above and fill in spaces A-N to identify the pictures below them.

A: _____	B: _____	C: _____
		

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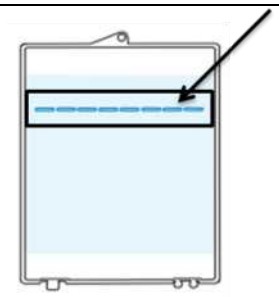
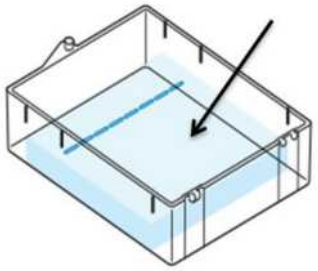
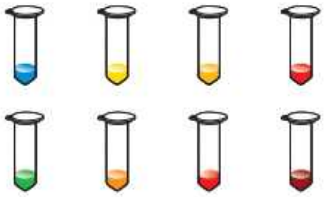
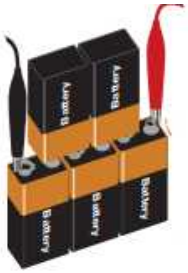
		
G: _____	H: _____ I: _____	J: _____
		
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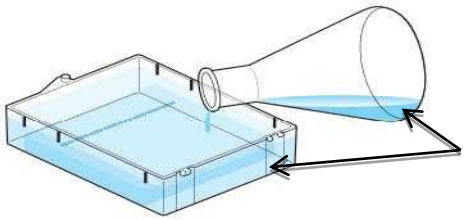
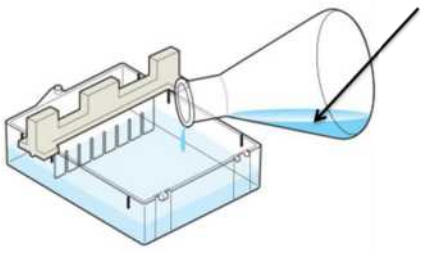
		
M: _____	N: _____	
		

Lesson 1 Electrophoresis & Genetic Trait Exploration

Determining Your Procedure

Now that you are familiar with the different components with which you will be working, how should you move forward with your agarose gel electrophoresis procedure? What are some things you should consider related to your sample, putting together the electrophoresis apparatus, and making and preparing your gel for samples? How long do you think the gel should be run and how will you know when it is done? What might you need to do with the gel once the samples are done running? Using the space below and on the back of this page, work with your lab mates to number and describe several steps, in logical order, you would use to complete this procedure.

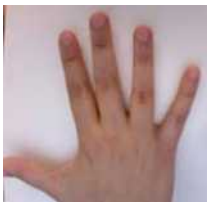
Lesson 1 Electrophoresis & Genetic Trait Exploration

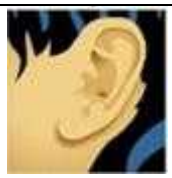
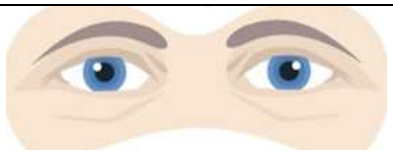
Human Genetic Traits Inventory

(Adapted from Biology Department, Grand Valley State University)

In simple Mendelian inheritance, traits are controlled by a single gene where one allele is received from each parent. If an allele is dominant, the trait will be expressed even if only one copy is inherited from the parents. If an allele is recessive, two copies must be inherited for the trait to be expressed. Those individual who inherit two matching alleles are called homozygous while those who inherit two non-matching alleles are termed heterozygous. The genotype is the total representation of a person's genes, and the phenotype is the expression of those genes. Take a look at the following example for tongue rolling.

The ability to *roll your tongue* upward is a dominant phenotype which can be represented by the genotypes RR (homozygous dominant) and Rr (heterozygous). The inability to roll your tongue would be the recessive phenotype, represented by the genotype rr (homozygous recessive).

Trait	Dominant Form	Recessive Form
1. Tongue Rolling *(Image source: drgcdms.blogspot.com)*	 RR or Rr	 rr
2. Facial Dimples *(Image source: reachoutmichigan.org)*	 DD or Dd	 dd
3. Bent Little Finger *(Image source: carolguze.com)*	 BB or Bb	 bb

4. Free Ear Lobes *(Image source: drgcdms.blogspot.com)*	 FF or Ff	 ff
5. Mid-digital Hair *(Image source: drgcdms.blogspot.com)*	 HH or Hh	 hh
6. Hand Clasping *(Image source: learn.genetics.utah.edu)*	 LL or Ll	 ll
7. Widows Peak *(Image source: drgcdms.blogspot.com)*	 WW or Ww	 ww
8. Hitchhiker's Thumb *(Image source: wikispaces.psu.edu)*	 TT or Tt	 tt
9. Cleft Chin *(Image Source: genetics.thetech.org)*	 CC or Cc	 cc
10. Eye Color *(Image source: bbc.co.uk)*	 MM or Mm	 mm

Human Genetic Traits Inventory

After reviewing the phenotypes on the previous page complete the inventory below by determining your own phenotype and genotype as best you can. You may need to think about the traits in your parents to help determine the details. We will then take a look at the total inventory for our class when everyone is complete.

Trait	Symbol	Dominant Phenotype	Your Phenotype	Your Genotype	Number of Phenotypes in Class	
					Dominant	Recessive
1. Tongue Rolling	R, r	Rolled				
2. Facial Dimples	D, d	Present				
3. Bent Little Finger	B, b	Bent				
4. Free Ear Lobes	F, f	Free				
5. Mid-digital Hair	H, h	Present				
6. Hand Clasping	L, l	Top Left Thumb				
7. Widow's Peak	W, w	Present				
8. Hitchhiker's Thumb	T, t	Absent				
9. Cleft Chin	C, c	Present				
10. Eye Color	M, m	Melanin (Brown)				

Lesson 1 Electrophoresis & Genetic Trait Exploration

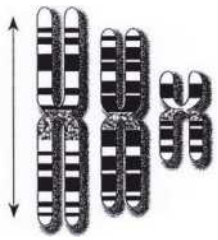
Human Karyotype Exercise

(Adapted from Biozone International 1995-2003)

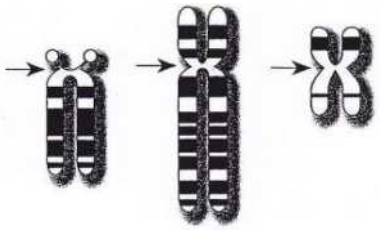
Normal human cells contain 23 pairs of chromosomes, one chromosome of each pair coming from each parent. However, during the production of gametes, occasionally genetic material is lost or rearranged. Many times these changes are so severe that either fertilization does not occur or the resulting embryo will not survive. However, approximately one in 156 live births has some kind of chromosomal abnormality.

An example of a chromosome abnormality is Down Syndrome in which there is an extra chromosome 21. This occurs in 1 in 700 births in the U.S. and this condition is associated with mental retardation, characteristic facial features, heart defects, respiratory infections, leukemia, and Alzheimer's disease. For these cases, a karyotype may offer information about abnormalities related to chromosome structure and number, in addition to the sex of the fetus.

Karyotypes are created by taking a sample of blood, or uterine fluid for fetuses, and staining and photographing the chromosomes from a cell. A cell undergoing mitosis is required, preferably in metaphase, so that the chromosomes are condensed and visible under a microscope just after replication. The photograph is enlarged and cut up into individual chromosomes which are then arranged according to homologous pairs and ordered by size (placing the sex chromosomes last; the X being the larger, and Y being the smaller of the sex chromosomes). Homologous pairs can be identified based on chromosome length, centromere position, banding pattern, and the presence of satellite endings.



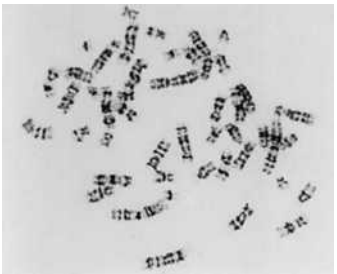
Chromosome Length



Centromere Position

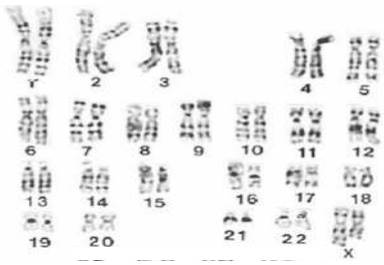
Banding Pattern

Satellite Endings



Spread of Human Chromosomes

(Source: Foundation for Research in Genetics & Endocrinology)



Karyotype of Human Chromosomes

(Source: Brazilian Journal of Genetics, Vol. 20, No. 3, 1997)

For this exercise, you will be working with a partner to create your own karyotype. From the chromosome spread on the next sheet, carefully cut out each chromosome. Find the homologous pairs by matching length, the position of the centromere, and the banding pattern. Arrange the pairs on the karyotype form below in order from longest to shortest, placing the sex chromosomes at the end. Tape lightly at first. Once you are confident that you have them placed correctly, tape them down more securely. Can you answer the following questions about your sample?

d) What is the sex of this individual: Male or Female ? (circle one)

e) Determine the state of the chromosomal arrangement: Normal or Abnormal ? (circle one)

f) If the arrangement is abnormal, state in what way: _____

1	2	3
A		

4	5
B	

6	7	8	9	10	11	12
C						

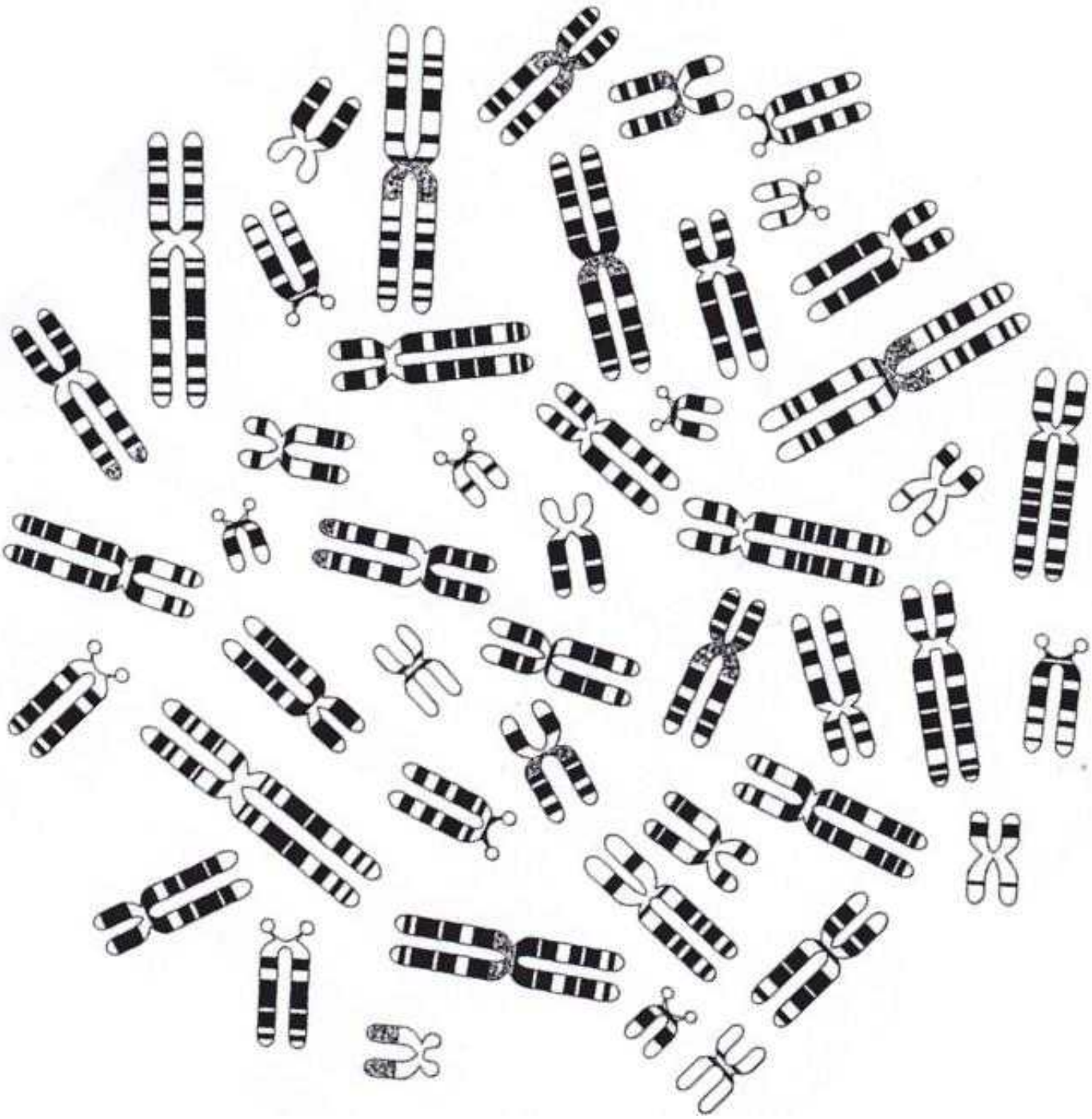
13	14	15
D		

16	17	18
E		

19	20
F	

21	22
G	

X	X or Y
Sex	



Lesson 2 Dye Electrophoresis & Gene Exercises

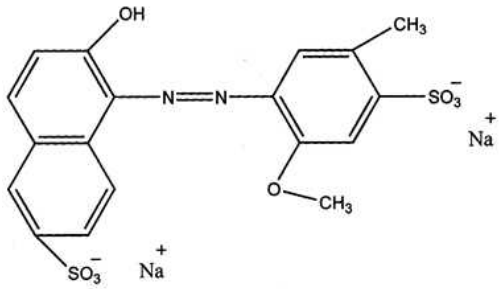
Dye Agarose Electrophoresis Procedure

(Adapted from Biotechnology Explorer, STEM Electrophoresis Kit, 166-5080EDU, Bio-Rad)

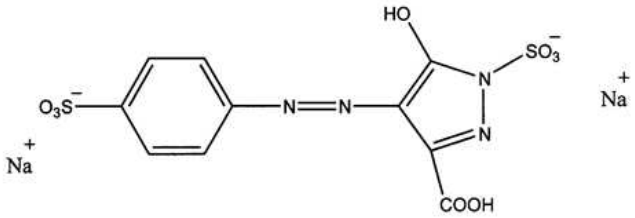
Reagents (provided by kit)	**Equipment & Supplies (provided by kit)**	**Shared Items (not provided by kit)**
Dye extraction solution, 25 ml 1 bottle	2 ml microcentrifuge tubes, 72 tubes	9 V batteries, 6–10
Blue 1 reference dye, 150 µl, 1 vial	Hinged plastic boxes, 1 box	Plastic rulers or plastic card to cut gels, 2
Yellow 5 reference dye, 150 µl, 1 vial	Paper clips, 4	2–20 µl adjustable-volume micropipette or 10 µl fixed-volume micropipette, 2
Yellow 6 reference dye, 150 µl, 1 vial	Black alligator clip leads, 2	2–200 µl pipet tips, 1,000/bag, 1 bag
Red 40 reference dye, 150 µl, 1 vial	Red alligator clips leads, 2	Eyedroppers or 100–1,000 µl adjustable-volume micropipette, 2; or disposable plastic transfer pipettes (DPTPs), 1 box; or 100–1,000 µl pipet tips, 1 bag
Electrophoresis buffer, 50x TAE, 100 ml, 1 bottle	8-well combs, 2	Marking pen, 2
Molecular biology grade agarose, 5 g, 1 bottle		Plastic cups or small beakers, 8
		Microwave oven or hot plate, 1
		Balance, 1
		Distilled water, 1 liter
		500 ml Erlenmeyer flask for microwaving agarose, 1
		Candies with a variety of color coatings variable (M&M's, Skittles, or Kool-Aid drink mixes)
		Optional
		Microcentrifuge or mini centrifuge, 1
		Digital camera for imaging gels, 1
		Microcentrifuge tube racks, 8

Structure of Reference Dyes

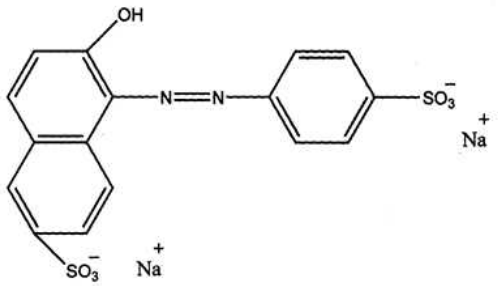
The Structure of Red 40



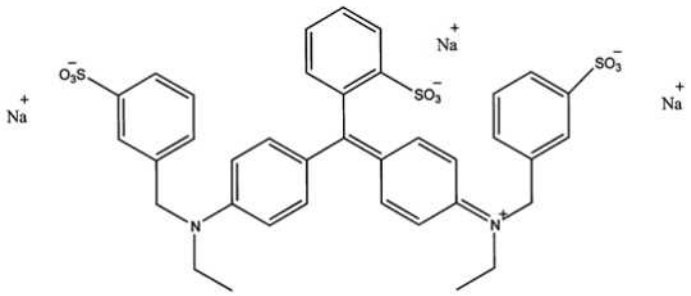
The Structure of Yellow 5



The Structure of Yellow 6



The Structure of Blue 1

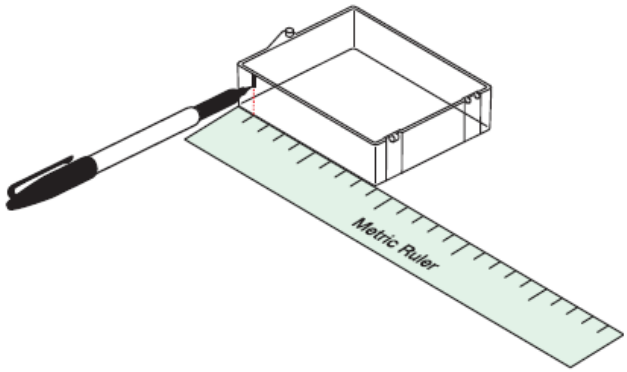


Unknown Sample #: _____

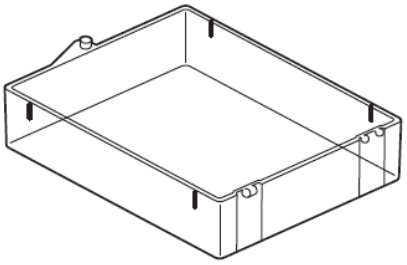
Hypothesis: _____

Part I: Prepare the gel boxes

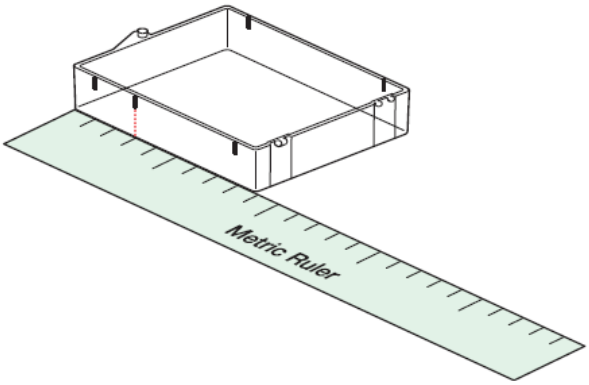
1. Measure a distance 1 cm from the end of the box on the longest side, and with a marking pen, and make a dash on the outside of the box.



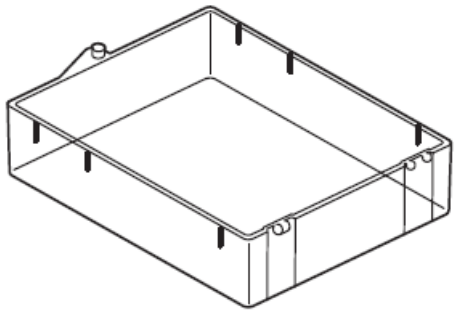
2. Repeat step 2 so that you have a mark 1 cm from the end of each of the longest sides of the box.



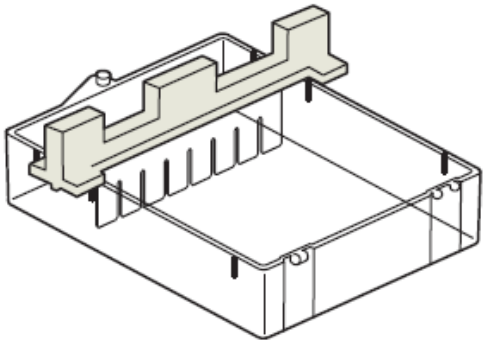
3. Measure a distance 3 cm from the end of the box on the longest side, and with a marking pen, and make a dash on the outside of the box.



4. Repeat step 4 on the opposite side of the box. When you have finished, each side of the box should have three marks.

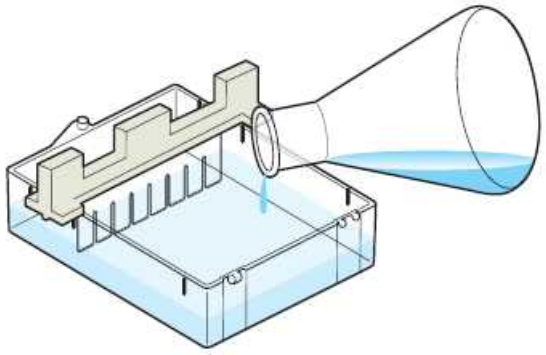


5. Place your 8-well comb on the marks that are 3 cm from the end. Make sure that the comb is centered so that none of the clear plastic well-formers touch the plastic box and that the comb is straight across the box.

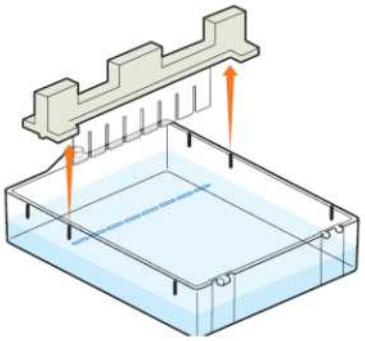


6. Carefully pour 50 ml of molten agarose into the box and allow the gel to solidify for 10–20 min. The gel will appear cloudy, or opaque, when ready to use.

   CAUTION: Always were protective gloves, goggles, and lab coat while preparing and casting agarose gels. Molten agarose or the flasks containing hot agarose can cause severe burns if allowed to contact skin.

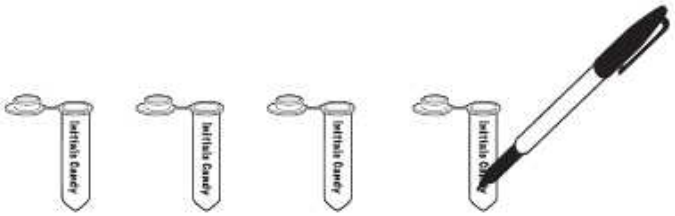


7. Carefully remove the comb from the solidified gel by pulling gently in an upward direction.



Part II: Dye extraction from candies (this has already been completed for you)

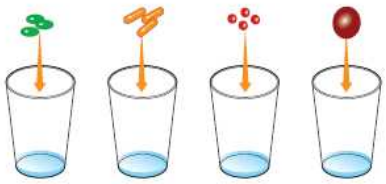
1. Label the four microcentrifuge tubes with the names and colors of the candies you are using.



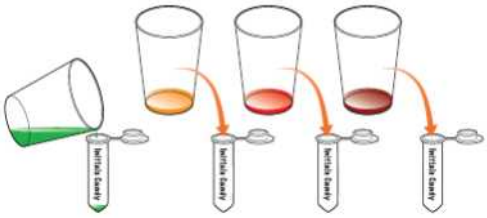
2. Label four cups with the names and colors of the candies you are using.

3. Using an eyedropper or pipet add 0.5 ml of dye extraction solution to each cup. Use the volume marks on the 2 ml microcentrifuge tube to measure the correct volume.

4. Place your candy into the appropriately labeled cup and swirl the candy in the dye extraction solution. If using a candy such as M&M'S or Skittles, just dissolve the color coating off until you get to the white layer of the candy. For all other candies, try to get as dark a solution of dye as possible.



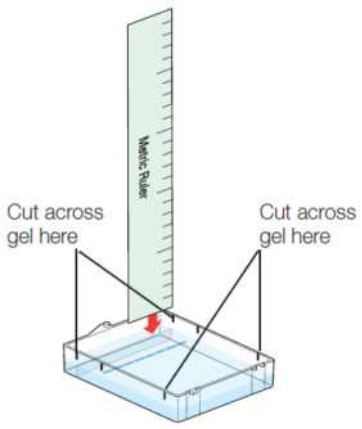
5. Remove your candy from the cup. Pour the solution containing the dissolved colored candy coating into the appropriately labeled microcentrifuge tube.



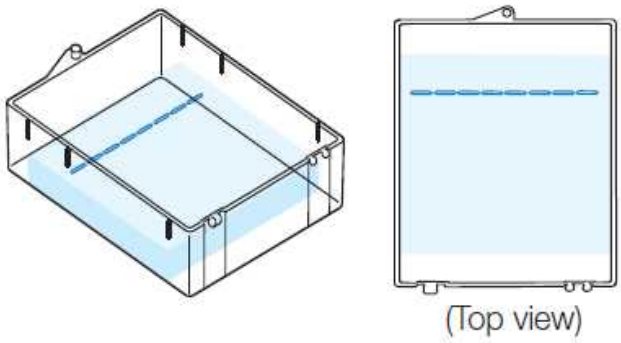
Part III: Agarose Gel Electrophoresis

1. Obtain your agarose gel in the plastic chamber. If you stored your gel after preparing it, pour off the 25 ml of 1x TAE buffer.

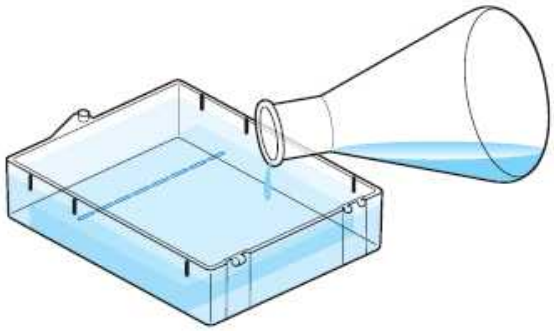
2. Using your ruler and following the marks you made one centimeter from the end of the box, cut a slab off the end of the gel using the end of a ruler. Press straight down through the gel to the box — do not slice across the gel. Loosen the slab by sliding the ruler between the end of the gel and the box end, then lift out the slab and discard.



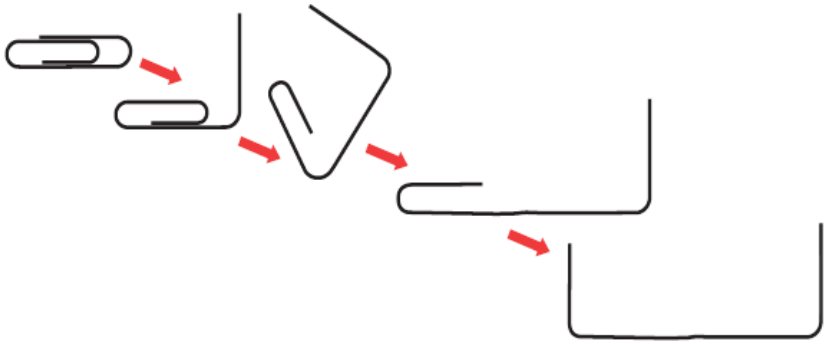
3. Repeat at the other end of the gel.



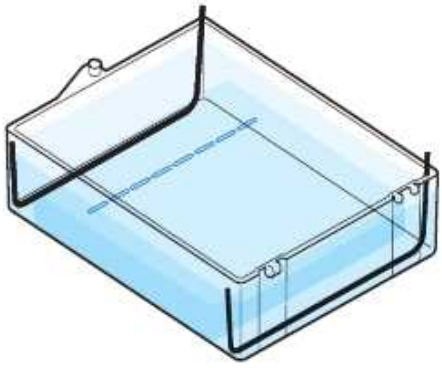
4. Add 55 ml of 1x TAE buffer to the box.



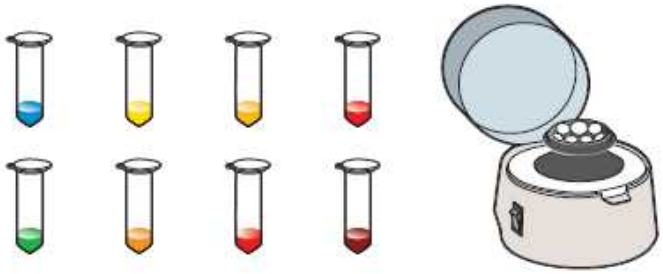
5. Construct your electrodes from two paper clips. Carefully straighten the paper clip and bend the two ends so they are perpendicular to the rest of the clip. Place your completed electrode on a flat surface. If it does not lie flat (in other words, if one of the angled pieces is not in the same plane as the rest of the electrode), hold the two ends and twist gently until the electrode will lie flat. The longer end will stick above the gel box – this is where you will attach the alligator clip.



6. Place the electrodes into the gel box with the long ends on the same side. The electrodes should be as close to the end of the box as possible (as far away from the gel as possible).



7. Prepare your extracted candy dye samples and reference dyes. If a centrifuge is available, pulse spin the microcentrifuge tubes in the centrifuge to bring all the liquid to the bottom of the tube and to settle any insoluble particles. Spin down your dye standard samples as well, if needed.



8. Using a separate tip for each sample, load 10 µl of each sample into 8 wells of the gel in the following order:

Lane 1: Blue 1 reference dye

Lane 2: Yellow 5 reference dye

Lane 3: Yellow 6 reference dye

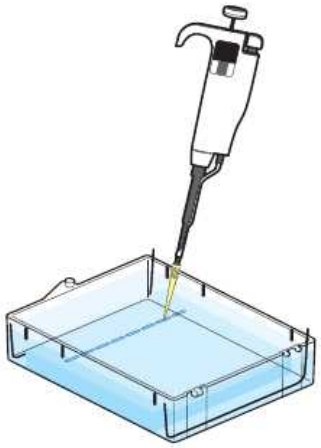
Lane 4: Red 40 reference dye

Lane 5: Candy 1 dye extract

Lane 6: Candy 2 dye extract

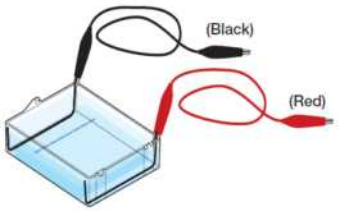
Lane 7: Candy 3 dye extract

Lane 8: Candy 4 dye extract

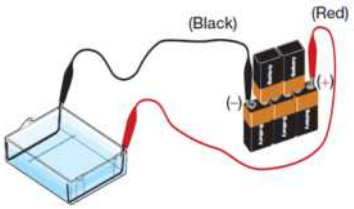


9. Assemble your battery tower by connecting negative nodes to positive nodes.

10. Attach the black alligator clip to the long end of the paper clip and box at the end of the box closest to the sample wells. Make sure the paper clip still remains on the bottom of the gel box under the buffer. Repeat the process for the red alligator clip and the electrode at the other end of the box.



11. When you are ready to begin your electrophoresis run, attach the free black alligator clip on your lead to the (–) terminal of your battery tower and the free red alligator clip on your lead to the (+) of your battery tower. You should notice bubbles coming off of the paper clip electrodes if the circuit is complete.



12. Allow your gel to run for 20 min. Disconnect the red and black alligator clips from the battery tower.

13. Optional: Take a photograph of the gel for your records.

Unknown Sample #: _____

Results: _____

Lesson 2 Dye Electrophoresis & Gene Exercises

Homework: Genetic Traits Video Questions

(Adapted from 23andMe.com)

- Visit the following website: www.23andMe.com/gen101/
- Watch the following videos and answer the questions below: What Are Genes? What are SNPs? Where Do Your Genes Come From? What Are Phenotypes?

What Are Genes?

1. Where do most of the genes reside in your body? _____
2. "DNA is a double-stranded molecule composed of _____, _____, and four different bases_____, _____, _____, and _____."
3. "Most genes are recipes for making specific _____."
4. "Gene _____ turn different genes on and off in different cells to control cell function."
5. How many pairs of chromosomes do humans have? _____
6. What percentage of the DNA in your chromosomes do you share with chimpanzees? _____ . Other humans? _____

What Are SNPs?

1. An entire set of 23 human chromosomes is called a _____.
2. Variation at a single base pair is called a SNP, or _____ _____ _____.
3. An SNP is created when a single base pair is _____, _____, or _____.
4. True or False. SNPs account for the genetic variation between you and other humans.

5. True or False. Many SNPs lead to no observable differences between people at all.

6. True or False. SNPs can be used to trace ancestry.

Where do Your Genes come from?

1. You inherit one _____ of chromosomes from each _____, which together are called _____ chromosomes.

2. Most cells contain two sets of chromosomes, but _____ and _____ cells each contain only one set of chromosomes.

3. Your siblings also receive one set of genes from each of your parents, but not necessarily the same combination of genes unless you are _____ _____.

4. When homologous chromosomes exchange DNA by crossing over, this is called genetic _____.

5. Ancestry along your father's line is easier to trace through the _____, and through your mother's line it is easier to trace through the _____.

What Are Phenotypes?

1. Your _____ _____ traits, also called your phenotype, results from the interaction between your genes and the environment.

2. Give two examples of phenotypes which are controlled by the interaction between your genes and the environment, such as diet and exercise; 1) _____and 2) _____.

3. True or False. Not much is known about how your genes affect your personality.

Lesson 3 Class Discussion of Electrophoresis and Genetic Traits

Review of DNA and Genes

(Adapted from Genetic Science Learning Center and Bio-Rad Biotechnology Explorer

Image Source: www.bio-rad.com)

Review Questions

1. What is DNA?

2. What does "DNA" stand for?

3. What is the four-letter DNA alphabet and what are the special rules by which the alphabet pieces bond together?

4. What is a gene?

5. What are genes made of?

6. For what molecule do genes contain the instructions for building?

7. What is a chromosome?

8. How many chromosomes does a human cell hold?

9. How are the human sex chromosomes labeled?

10. What provides the "blueprint" for making a protein?

11. What is heredity?

12. Why aren't children identical to either one of their parents?

13. In humans, how many chromosomes does each parent pass on to their offspring?

14. Does the second child in a family inherit the exact same chromosomes as the first? Do both babies have a complete set?

15. What is a trait?

16. Give an example of how an environmental factor can influence a trait.

17. Briefly explain how the Hitchhiker's Thumb trait is determined using the following words: allele, dominant, recessive, homozygous, heterozygous. You may draw pictures if you wish.

Lesson 3 Class Discussion of Electrophoresis and Genetic Traits

Introduction to Electrophoresis

(Adapted from Biotechnology Explorer, STEM Electrophoresis Kit, 166-5080EDU, Bio-Rad)

1. When you analyzed the results of your gel, did any of your experimental samples contain dyes that did not match the four reference dyes? For example, did any of your samples produce:

    a. Dyes that are a different size than any of the standard bands?

    b. Dyes that are a different color than any of the standard bands?

    c. More than one color band?

    d. Dyes that moved a different distance compared to the standard bands?

2. We powered our electrophoresis with 9 V batteries connected in a series (multiple batteries with negative electrodes connected to positive electrodes to form a chain). For batteries connected in series, the total voltage is equal to the sum of the voltages of the individual batteries.

    a. How many batteries did we use? _____

    b. What would be the voltage if we used three batteries in series? _____ volts

    c. What if we used five batteries? _____ volts

    d. Would we expect our samples to migrate faster through the gel if we used three batteries or five batteries? Why? What might be some disadvantages of using a higher voltage for the power supply?

3. For these experiments, we used a 1% agarose gel to separate the dyes. How do you think the results would differ if we used a 3% agarose gel? A 0.8% agarose gel?

4. What are some properties that need to be considered when picking a material to construct an electrophoresis box?

5. In this kit, you used zinc-coated steel paper clips to construct electrodes. Commercially-made gel boxes use platinum wire for electrodes. What are the characteristics needed for a good electrode?

6. Which direction would you expect a dye to run if it has no charge?

7. Calculate the rate that each dye moves in the gel.

    a. Using the photograph of your gel, measure the distance each reference dye moved from the wells in cm

    b. Divide the distance traveled by each reference dye by the time that you ran your gel (in minutes)

    c. Which dye has the fastest rate of movement in cm/min?

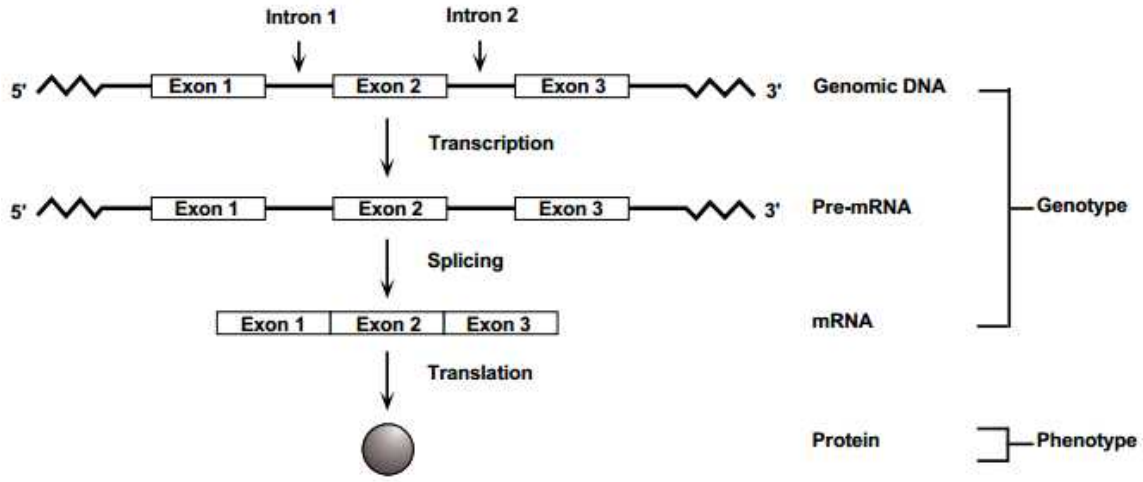
    d. Why might the distance travelled differ between the dyes?

DNA Processing

As we just reviewed, each human carries 23 pairs or 46 single chromosomes (23 from the mother and 23 from the father). It is estimated that these 46 chromosomes contain approximately 30,000 – 50,000 genes. Each chromosome contains a series of specific genes, dependent on its size. Each of the homologous chromosome pairs contains similar genes.

Each gene holds the code for a particular protein. Interestingly, the 30,000 – 50,000 genes only comprise 5% of the total chromosomal DNA. The other 95% is noncoding DNA. This noncoding DNA is found not only between, but within genes, splitting them into segments. The exact function of the noncoding DNA is not known, although it is thought that noncoding DNA allows for the accumulation of mutations and variations within genomes.

When RNA is first transcribed from DNA, it contains both coding and noncoding sequences. While the RNA is still in the nucleus, the noncoding **introns** (**in = stay within the nucleus**) are removed from the RNA while the **exons** (**ex = exit the nucleus**) are spliced together to form the complete messenger RNA coding sequence for the protein (see image below). This process is called RNA splicing and is carried out by specialized enzymes called spliceosomes.



Introns often vary in size and sequence among individuals, while exons do not. This variation is thought to be the result of the differential accumulation of mutations in DNA throughout evolution. These mutations in our noncoding DNA are silently passed on to our descendants; we do not notice them because they do not affect our phenotypes. However, these differences in our DNA represent the molecular basis of DNA fingerprinting used in human identification and studies in population genetics.

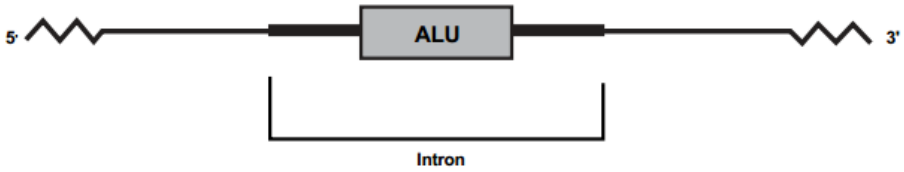
Lesson 4 Introduction to Human *Alu* PV92 & Isolation of Cheek Cell DNA

What Is Alu PV92?

(Adapted from DNA Learning Center, Cold Spring Harbor Laboratory and Bio-Rad Biotechnology Explorer)

Background

The function of noncoding pieces of DNA is not well understood; however, upon closer investigation scientists noticed several unique genetic elements repeated in a number of different locations. One of the first repeating elements found in all primates is called *Alu*. *Alu* repeats are approximately 300 base pairs long and are sometimes referred to as a "jumping gene" as *Alu* can get copied from one place to another place in the DNA via reverse transcriptase. It is believed that *Alu* first emerged a very long time ago, as many as 60 million years ago, as it now exists in all primates and is inserted in different locations across the genome of each species.



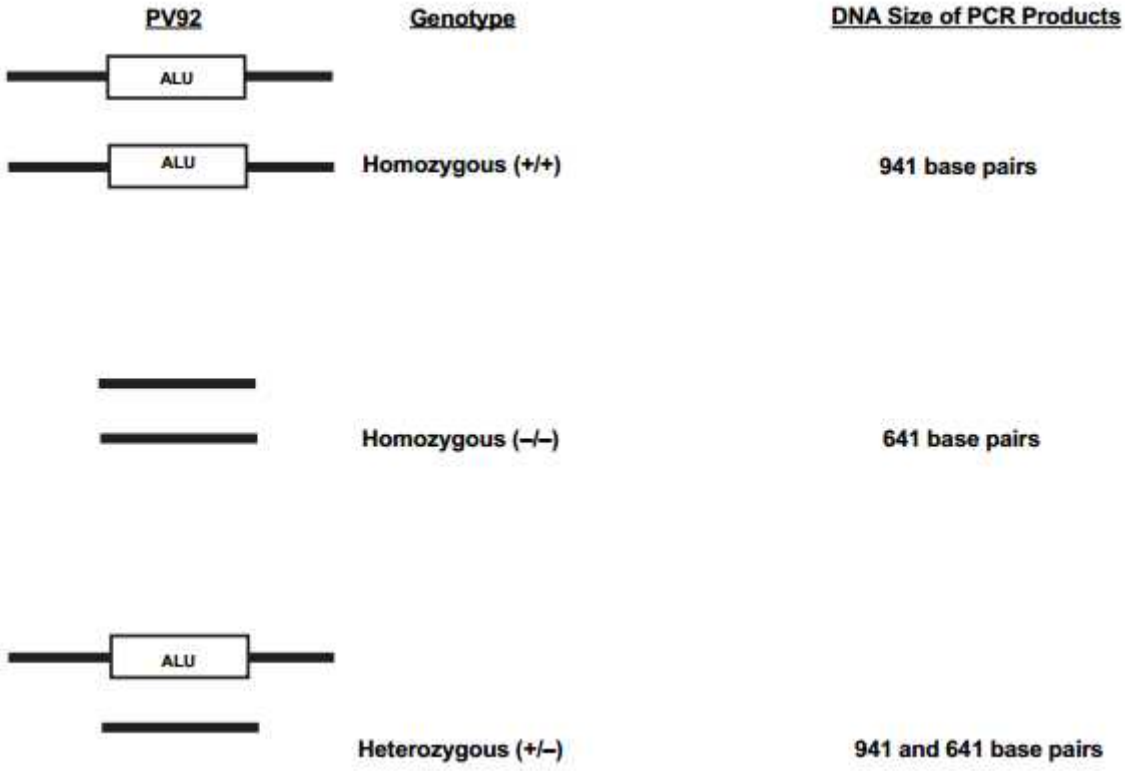
Most *Alu* insertions occur in non-coding regions of the DNA and as of yet have not been found to have any beneficial or adverse effects. However, some *Alu* insertions have been linked to certain conditions. For example, an *Alu* insertion in the NF-1 gene is responsible for neurofibromatosis I, and *Alu* insertions in introns of genes for tissue plasminogen activator (TPA) and angiotensin converter enzyme (ACE) are associated with heart disease.

Once an *Alu* inserts at a chromosome locus, it can copy itself for transposition, but there is no evidence that it is ever removed from a chromosome locus. So, each *Alu* insertion is stable through evolutionary time. Each is the "fossil" of a unique transposition event that occurred only once in primate evolution. Like genes, *Alu* insertions are inherited in a Mendelian fashion from parents to children. Thus, all primates showing an *Alu* insertion at a particular locus have inherited it from a common ancestor. This is called identity by descent.

Although many *Alu* insertions are found in all primate species, there are estimated 500-2000 insertions that are specific to the human genome. Many of the human-specific *Alu* insertions are "fixed" in the populations meaning that both of the paired chromosomes have an insertion at the same locus (position). However, a number of human-specific *Alu* insertions are dimorphic – an insertion may be present or absent on each of the paired chromosomes of different people. These dimorphic *Alu* insertions are the ones that have occurred most recently, within the last million years. These dimorphisms show differences in allele and genotype frequencies between modern populations and are tools for reconstructing human prehistory.

Current Investigation

Today we are going to look at *Alu* PV92 which is a human-specific *Alu* insertion on chromosome 16. It is dimorphic, meaning the element is present in some individuals and not others. The PV92 genetic system has only two alleles indicating the presence (+) or absence (-) of the *Alu* transposable element on each of the paired chromosomes. This results in three PV92 genotypes (++, +-, or --). The + and - alleles can be separated by size using gel electrophoresis.



Question

During this lab we are going to investigate the question; what is my *Alu* PV92 genotype?

Tasks:

1. Isolate DNA from our cheek cells.
2. Amplify DNA with PCR.
3. Analyze DNA using gel electrophoresis.
4. Report findings.

Lesson 4 Introduction to Human *Alu* PV92 & Isolation of Cheek Cell DNA

Isolation of Cheek Cell DNA Procedure

(Adapted from Bio-Rad Biotechnology Explorer Image Source: www.bio-rad.com)

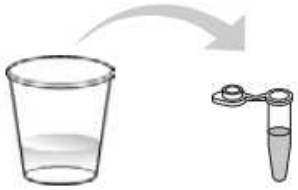
Reagents	**Equipment & Supplies (provided by kit)**	**Shared Items (not provided by kit)**
Saline Solution (0.9% NaCl), 10 ml	Foam micro test tube holders	P-20 micropipet
InstaGene matrix, 20 ml	Screw cap tubes	Pipet tips (filter type), 2-20 µl
	1.5 ml Micro test tubes, with attached caps	Permanent marker
		Biological waste container
		P-1000 or P-200 micropipet
		Pipet tips (filter type), 100-1000 µl or 20-200 µl
		Water baths (56 and 100 °C)
		Microcentrifuge or mini centrifuge
		Vortexer

Procedure

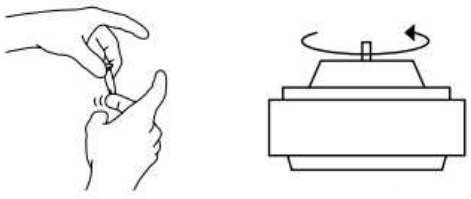
1. Each member of your team should have 1 screw cap tube containing 200 µl of InstaGene matrix, 1.5 ml micro test tube, and a cup containing 10 ml of 0.9% saline solution. Label one of each tube and a cup with your initials.

2. Do not throw away the saline solution after completing this step. Pour the saline from the cup into your mouth. Rinse vigorously for 30 seconds. Expel the saline back into the cup.

3. Set a P-1000 micropipet to 1,000 μl and transfer 1 ml of your oral rinse into the micro test tube with your initials. If no P-1000 is available, carefully pour ~ 1 ml of your swished saline into the micro test tube (use the markings on the side of the micro test tube to estimate 1 ml).



4. Spin your tube in a balanced centrifuge for 2 minutes at full speed. When the centrifuge has completely stopped, remove your tube. You should be able to see a pellet of whitish cells a t the bottom of the tube. Ideally, the pellet should be about the size of a match head. If you can't see your pellet, or your pellet is too small, pour off the saline supernatant, add more of your saline rinse, and spin again.



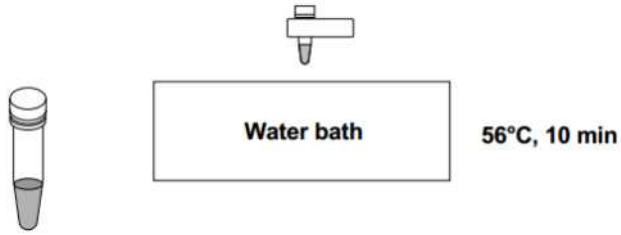
5. Pour off the supernatant and discard. Taking care not to lose your cell pellet, carefully blot your micro test tube on a tissue or paper towel. It's ok for a small amount of saline (~50 μl, about the same size as your pellet) to remain in the bottom of the tube.

6. Resuspend the pellet thoroughly by vortexing or flicking the tubes until no cell clumps remain.

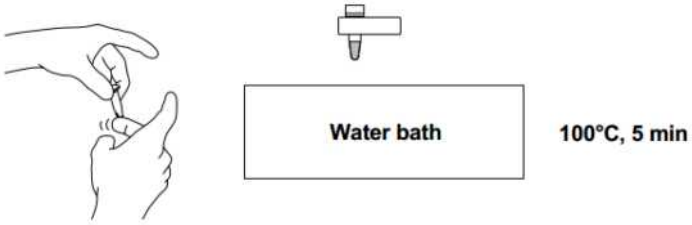
7. Using an adjustable volume micropipette set to 20 µl, transfer your resuspended cells into the screw cap tube containing the InstaGene with your initials. You may need to use the pipet a few times to transfer all of your cells.

8. Screw the caps tightly on the tubes. Shake or vortex the mix the contents.

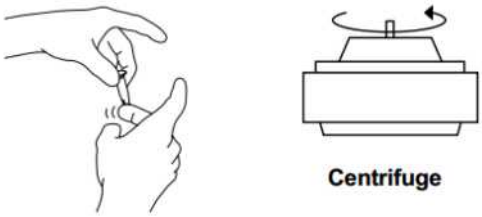
9. Place the tubes in the foam micro test-tube holder. When all members of your team have collected their samples, float the holder of tubes in a 56 °C water bath for 10 minutes. At the halfway point (5 minutes), shake or vortex your tubes several times. Place the tubes back in to the water bath for the remaining 5 minutes.



10. Remove the tubes from the water bath and shake them several times. Now float the holder with tubes in a 100 °C water bath for 5 minutes.



11. Remove the tubes from the 100 °C water bath and shake or vortex several times to resuspend the sample. Place the eight tubes in a balanced arrangement in a centrifuge. Pellet the matrix by spinning for 5 minutes at 6,000 x g (or 10 minutes at 2,000 x g).



12. Store your screw cap tube in the refrigerator until the next laboratory period.

Lesson 5 PCR Amplification of DNA

PCR Procedure

(Adapted from Bio-Rad Biotechnology Explorer Image Source: www.bio-rad.com)

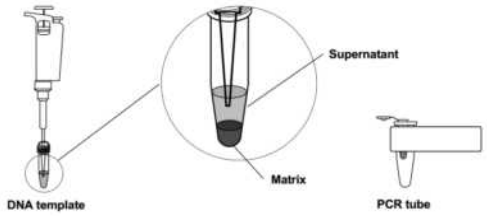
Reagents	**Equipment & Supplies (provided by kit)**	**Shared Items (not provided by kit)**
Human DNA sample	Foam micro test tube holders	P-20 micropipet
Complete master mix (with primers) on ice	PCR tubes	Pipet tips (filter type), 2-20 µl
	1.5 ml Micro test tubes, capless	Ice bucket with ice
		Permanent marker
		Biological waste container
		Microcentrifuge or mini centrifuge
		Thermal Cycler

Procedure

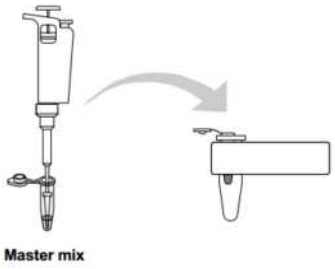
1. Obtain your screw cap tube that contains your genomic DNA template from the refrigerator. Centrifuge your tubes for 2 minutes at 6,000 x g or for 5 minutes at 2,000 x g.

2. Each member of the team should obtain a PCR tube and capless micro test tube. Label each PCR tube on the side of the tube with your initials and place the PCR tube into the capless micro test tube as shown. Place the PCR tube in the foam micro test tube holder.

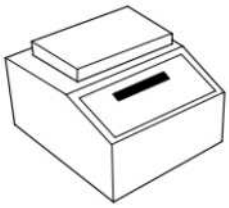
3. Transfer 20 µl of your DNA template from the supernatant in your screw cap tube into the bottom of the PCR tube. **Do not transfer any of the matrix beads into the PCR reaction because they will inhibit the PCR reaction.**



4. Locate the tube of yellow PCR master mix (labeled "Master") in your ice bucket. Transfer 20 µl of the master mix into your PCR tube. Mix by pipetting up and down 2-3 times. Cap the PCR tube tightly and keep it on ice until instructed to proceed to the next step. Avoid bubbles, especially in the bottom of the tubes.



5. Remove your PCR tube from the capless micro test tube and place the tube in the thermal cycler.



6. When all of the PCR samples are in the thermal cycler, the teacher will begin the PCR reaction. The teacher will also include the electrophoresis controls in this reaction. The reaction will undergo 40 cycles of amplification, which will take approximately 3 hours.

Cycle	Step	Function	Temperature	Time
1	Step 1	Pre-denaturation	94°C	2 minutes
	Repeat 1 time			
2	Step 1	Denature	94°C	1 minute
	Step 2	Anneal	60°C	1 minute
	Step 3	Extend	72°C	2 minutes
	Repeat 40 times			
3	Step 1	Final Extension	72°C	10 minutes
	Repeat 1 time			
*	Step 1	Hold	4°C	Infinity

Lesson 5 PCR Amplification of DNA

Understanding PCR

(Adapted from Bio-Rad Biotechnology Explorer, www.dnalc.org, and University of Utah Health Sciences
Image Source: www.bio-rad.com)

Introduction

Polymerase chain reaction (PCR) is a technique that was developed in 1983 by Kary Mullis, and is now universally used by scientists to quickly amplify segments of DNA. Amplification means to create millions of copies of specific regions of DNA without resorting to other labor intensive cloning procedures. Review the following videos to learn how PCR works:

http://www.dnalc.org/resources/animations/pcr.html

http://learn.genetics.utah.edu/content/labs/pcr/

The amplification takes place in a reaction mixture. This mixture must contain several key ingredients for the PCR to take place effectively. Do you know the purpose of each component in the reaction mixture? Fill in your answers below:

1. DNA template - _____.
2. Individual deoxynucleotides – _____.
3. DNA polymerase – _____.
4. Magnesium ions – _____.
5. Oligonucleotide primers – _____

   _____.
6. Salt buffer – _____

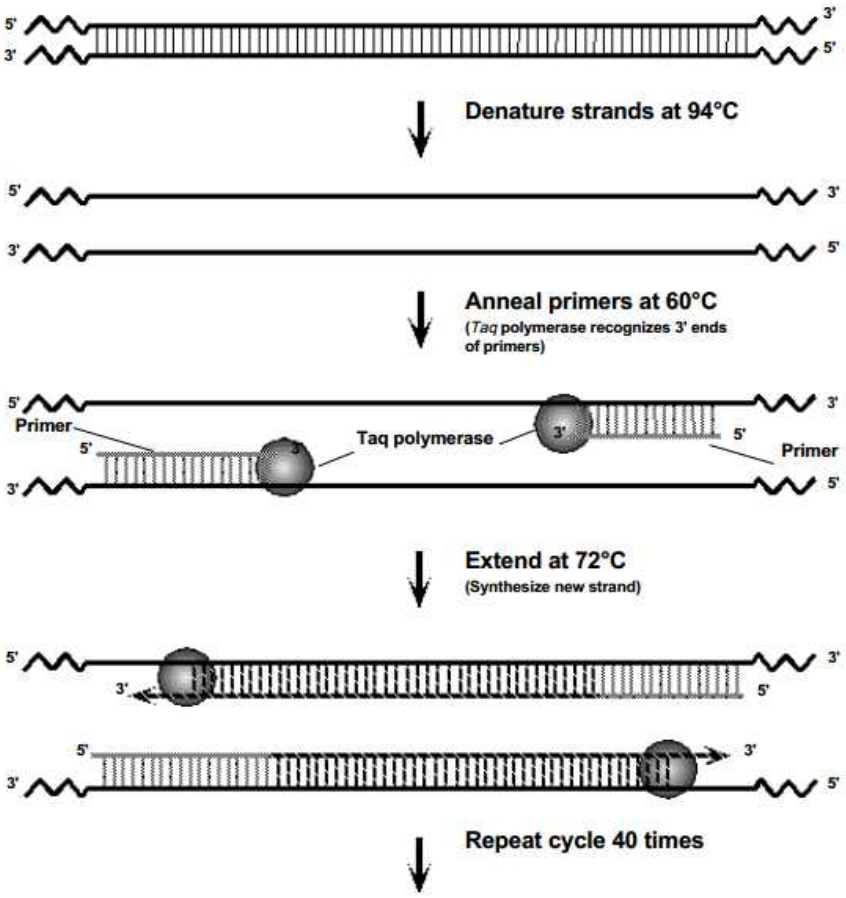
   _____.

If you recall, in this experiment, we are interested in making copies of the specific *Alu* PV92 region on chromosome 16. The template DNA is extracted from the cheek cell isolation you performed earlier in the lab. The primers for this reaction are as follows:

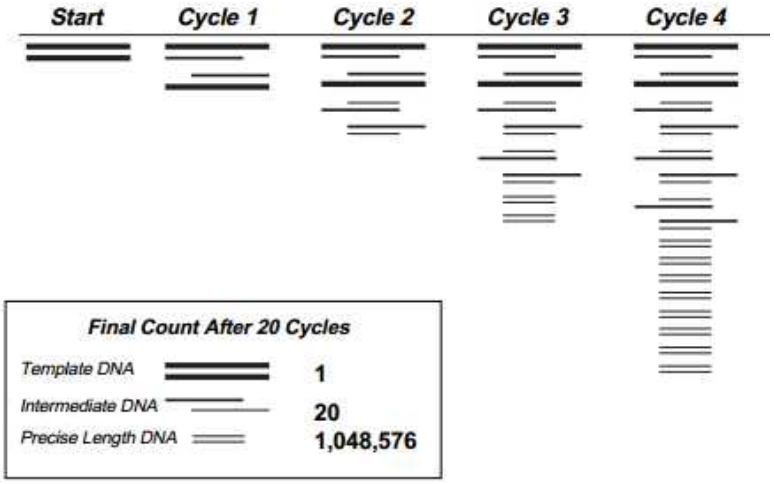
Forward primer: 5' GGATCTCAGGGTGGGTGGCAATGCT 3'

Reverse primer: 5' GAAAGGCAAGCTACCAGAAGCCCCAA 3'

The Reaction



After each of the 40 cycles of PCR, the DNA segment of interest will grow in copy number exponentially (X^n). Using this formula, how many copies would you have after 2 cycles; after 4 cycles?



Lesson 6 DNA Analysis by Gel Electrophoresis

DNA Electrophoresis Procedure

(Adapted from Bio-Rad Biotechnology Explorer Image Source: www.bio-rad.org)

Reagents	**Equipment & Supplies (provided by kit)**	**Shared Items (not provided by kit)**
Prepared PCR samples	Foam micro test tube holders	P-20 micropipet
PV92 XC DNA loading dye	Gel staining tray	Pipet tips (filter type), 2-20 µl
MMR (DNA standards)		Permanent marker
Fast Blast DNA stain, 1x or 100x solution		Gel box and power supply
1x TAE electrophoresis buffer		Biological waste container
Amplified positive control samples		Microcentrifuge or mini centrifuge
PV92 homozygous (+/+)		**For Quick Staining Protocol**
PV92 homozygous (-/-)		Warm tap water for detaining
PV92 heterozygous (+/-)		Large containers for destaining
Molten agarose, 50 ml		**Optional**
		Gel support film
		Clear acetate sheets for tracing gels
		Rocking platform

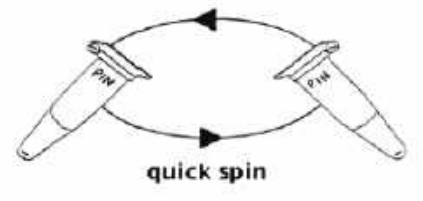
Part I: Prepare The Agarose Gels

The recommended gel concentration for this exercise is 1%, and the molten agarose gel has already been prepared to this concentration by adding 1 g of agarose to 100 ml of 1x TAE electrophoresis buffer. To make the electrophoresis gels, complete the following procedure:

1. Be sure the gel molding tray is constructed following your instructor's directions. Also ensure that it is setting on a level surface.
2. In an Erlenmeyer flask, obtain 50 mL of the prepared agarose gel. Slowly pour the agarose into the molding tray without creating bubbles. If bubbles do occur, you can use the tip of a plastic pipet tip to clear the bubbles off to the edge of the gel.
3. Once all the agarose is poured and bubbles are cleared, place the comb into the gel and allow it to cool.
4. The gel will become slightly cloudy once it has reached room temperature. At this point, the comb can be removed by gently moving it back and forth to loosen it from the gel. Pull it out slowly so as to not cause tears in your wells.

Part II: Gel Electrophoresis of Amplified PCR Samples

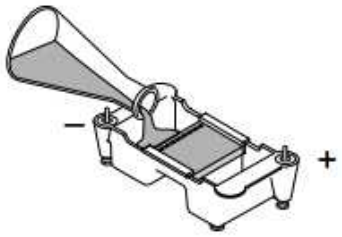
1. Remove your PCR samples from the refrigerator and place in the micro test tube holder. If a centrifuge is available, place the PCR tubes in the capless micro test tubes and pulse-spin the tubes (~ 3 seconds at 2,000 x g) to bring the condensation that formed on the lids to the bottom of the tubes.



2. Add 10 µl of PV92 XC loading dye to each PCR tube and mix gently.

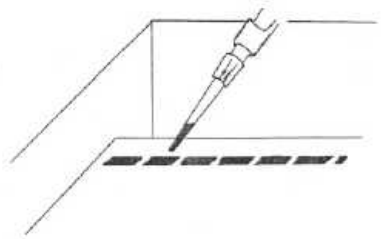
3. Ensure that your casting gel with the solidified gel in it is on the platform in the gel box. The wells should be at the cathode (-) end of the box, where the black lead is connected. If you haven't already, very carefully remove the comb from the gel by pulling straight up, slowly.

4. Pour ~ 250 ml of electrophoresis buffer into the electrophoresis chamber, until it just covers the wells.

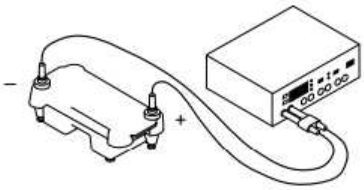


5. Using a clean tip for each sample, load the samples into the 8 wells of the gel in the following order:

Lane	Sample	Load Volume
1	MMR (DNA standard)	10 µl
2	Homozygous (+/+) control	10 µl
3	Homozygous (-/-) control	10 µl
4	Heterozygous (+/-) control	10 µl
5	Student 1:	20 µl
6	Student 2:	20 µl
7	Student 3:	20 µl
8	Student 4:	20 µl



6. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect the electrical leads to the power supply.



7. Turn on the power supply. Set it to 100 V and electrophorese the samples for 30 minutes.

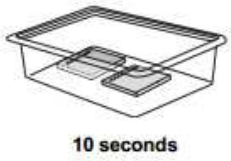
8. When electrophoresis is complete, turn off the power and remove the lid from the gel box.

Part III: Staining Your Gel

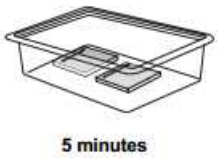
Warning: Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes.

1. *Stain Gels.* Carefully remove the gel tray and the gel from the gel box. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray. Pour approximately 120 ml of 100x stain into the staining tray. If necessary, add more 100x stain to completely submerge the gels. Stain the gels for 2 – 3 minutes, but not for more than 3 minutes. Using a funnel, pour the 100 x stain into a storage bottle and save it for future use. The stain can be reused at least 7 times.

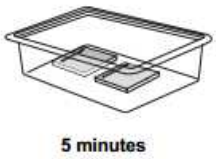
2. *Rinse Gels.* Transfer the gels into a large container containing 500 – 700 ml of clean, warm (40-55 °C) tap water. Gently shake the gels in the water for ~ 10 seconds to rinse.



3. *Wash Gels.* Transfer the gels into a second large container with 500 – 700 ml of clean, warm tap water. Gently rock or shake the gels on a rocking platform for 5 minutes. If no rocking platform is available, move the gels gently in the water once every minute.



4. *Wash Gels.* Perform a second wash as in step 3.



5. *Record Results.* Take a picture of your gel for analysis during the next class period.

Lesson 6 DNA Analysis by Gel Electrophoresis

Discussion Questions

(Adapted from Bio-Rad Biotechnology Explorer)

1. Explain the differences between an intron and an exon.

2. Explain how agarose electrophoresis separates DNA fragments. Why does a smaller DNA fragment move faster than a larger one?

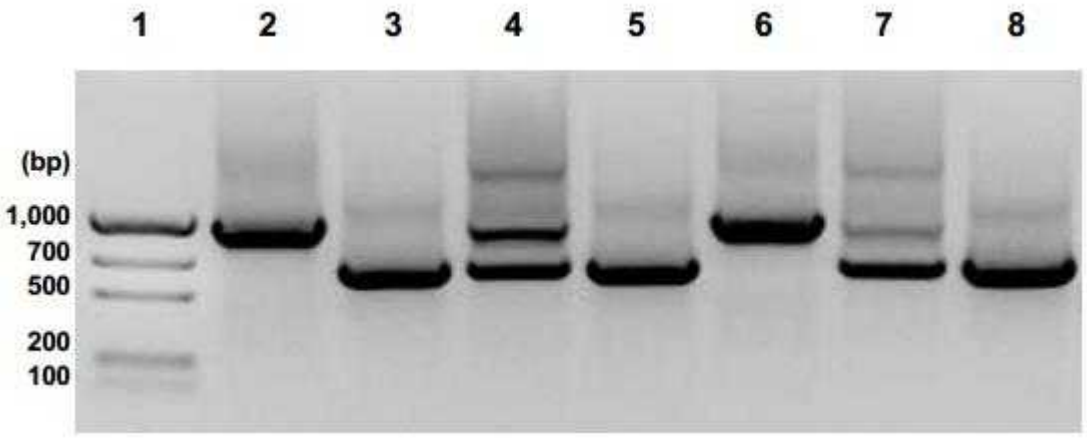
3. What kinds of controls are run in this experiment? Why are they important? Could others be used?

Lesson 7 Analysis and Interpretation of Your Results

Interpreting Your Gel

(Adapted from Dolan DNA Learning Center, Cold Spring Harbor Laboratory and Bio-Rad Biotechnology Explorer Image Source: www.bio-rad.org)

1. Determine your PV92 genotype. Practice with the gel below. Based on what you know about the *Alu* locus on chromosome 16, can you determine what genotype each lane displays? How do you know? Label each lane on the gel below and then use it as a key in determining the genotypes represented on your own gel photograph.



Other notes to keep in mind when analyzing your gel:

a. It is common to see a diffuse (fuzzy) band that runs ahead of the 121-bp marker. This is "primer dimer," an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. The presence of primer dimer, in the absence of other bands, confirms that the reaction contained all components necessary for amplification.

b. Additional faint bands at other positions occur when the primers bind to chromosomal loci other than the PV92 locus and give rise to "nonspecific" amplification products.

My sample shows band(s) at: _____

My *Alu* PV92 genotype is: _____

2. Why do the two possible PCR products differ in size by 300 base pairs?

3. Determine the observed genotype and allele frequencies for your class using the equations below. Determine what table best represents your data, draw it in the space below, and record your answers there.

$$\text{genotype frequency (\%)} = \frac{\text{number of students of X genotype}}{\text{total student samples}} \times 100$$

$$\text{allele frequency (\%)} = \frac{\text{number of X alleles}}{\text{total alleles in sample}} \times 100$$

Lesson 8 Molecular Techniques and Genetic Diseases/Disorders

Investigating Genetic Disorders

The medical field is now more capable of detecting genetic disorders through various testing techniques. Explore the links below to familiarize yourself with how PCR and agarose gel electrophoresis might assist medical experts in detecting different disorders. Also look through the following links to see if there are techniques in addition to electrophoresis that are of use in detecting genetic disorders.

https://www.inkling.com/read/robbins-cotran-pathologic-basis-of-disease-8th/chapter-5/molecular-diagnosis-of-genetic

http://advameddx.org/download/files/AdvaMedDx_DxInsights_FINAL(2).pdf

http://www.hhmi.org/biointeractive/human-genetics-new-guide-medicine

http://www.hhmi.org/biointeractive/medicine-genomic-era

http://www.hhmi.org/biointeractive/telltale-genes-charting-human-disease

Reflection

1. What are some examples of how gel electrophoresis can help detect a genetic disorder or disease?

2. What is another technique for detecting genetic disorders or diseases that was new to you? Please describe how the process works below.

3. In addition to determining genetic disorders, what might be other uses for running a gel electrophoresis?

Lesson 9 Pharmacogenomics

Pharmacogenomics Exploration

In the previous exercise, you ran an agarose gel electrophoresis in order to determine your genotype for the transposon *Alu* PV92. You could run an agarose gel electrophoresis to determine your genotype at any number of genetic locations for many different purposes. Review the following links to see how a person's genotype at specific single nucleotide polymorphisms (SNPs, which we explored at the beginning of this lesson) could influence the medical treatment he or she receives.

http://www.youtube.com/watch?v=-6TEfYZQZnw

http://learn.genetics.utah.edu/content/pharma/intro/

http://learn.genetics.utah.edu/content/pharma/snips/

http://learn.genetics.utah.edu/content/pharma/development/

Reflection

1. Give an example of how molecular techniques help personalize medical treatments. What are the benefits for tailoring medical treatments to a person's genotype for this particular disease? Are there any negative consequences?

2. In addition to designing medical treatments, what other reasons are there for knowing a person's genotype or genome?

3. What do you think about the challenges and issues in personalized medicine and Pharmacogenomics?

Lesson 10 Introduction to Genetic Based Diseases/Disorders

Genetic Disorders

The medical field has also been able to better diagnose and treat diseases because for many, we now better understand the genetic basis of the disease. A **genetic disorder** is a disease that is caused by an abnormality in an individual's DNA. Abnormalities can range from a small mutation in a single gene to the addition or subtraction of an entire chromosome or set of chromosomes. To learn more about different genetic disorders, browse through the following links:

http://www.biochemistry.org/Portals/0/Education/Docs/BASC10_full.pdf

http://learn.genetics.utah.edu/content/disorders/

http://www.chg.duke.edu/diseases/disorders.html

http://www.yourgenesyourhealth.org/

Reflection

1. Which genetic disorder or disease is of interest to you; which might you be interested in exploring further? Please describe it below.

2. Was there a disorder or disease described here that you previously did not know had a genetic basis? What else was new or surprising to you?

3. What are the different types of genetic problems that might lead to the formation of a genetic disorder or disease? Do you understand the molecular mechanism by which they occur?

Lesson 11 Genetic Disorder/Disease Presentations

Presentation Guidelines

In your group, use any of the previous resources you've explored, and select a genetic disorder to learn more about and present to the rest of the class. You may select any disease listed on these sites. If there is one of interest to you that is not on this page, please check with your instructor first before researching the disease.

Your group will be presenting (10-15 minutes) to the class the following information:

How do people get the disease? Describe how the genetic mutation becomes incorporated into a person's genome, describe possible inheritance patterns.

What is the disease? Describe the genetic basis for the disease and what effects this genetic disorder has on the body and development.

What are the symptoms of the disease? Fully describe the direct effects of the genetic disorder, if not already covered in the areas above, and add any secondary effects and complications that may be experienced throughout a person's lifetime.

How do doctors diagnose the disease? Describe the characteristics doctors look for and the tests that may be run to determine diagnosis.

How is the disease treated? Describe the medications, therapies, and treatments for this disease, including (if provided) the dosages, timeline, costs, and possible side effects to be aware of related to the treatment.

Interesting facts about the disease? This category may include information related to how the disease was first discovered and characterized, how it got its name, and the incidence rate (i.e. "This disease occurs in about 1 out of every 100,000 people" or "10 children are diagnosed in the United States each year").

Your group must include at least **two scholarly sources** in addition to the website provided. Please be sure not to just plagiarize the recommended website. For assistance in identifying scholarly sources, please refer to: http://www.emich.edu/library/help/peerreview.php. Also, feel free to further explore http://learn.genetics.utah.edu to assist you in understanding any of the concepts you may encounter.

The **presentation** can take any form you like; PowerPoint, webpage, Prezi, song, video, or poster board, etc. But, please try to have some sort of visual. I prefer you <u>not</u> just get up and talk through each point.

Lesson 11 Genetic Disorder/Disease Presentations

Grading Rubric

(Reference: Rubric for Oral Presentations, New England Association of School and Colleges, Commission on Public Secondary Schools)

	Exceeds Standard	Meets Standard	Emerging	Attempt Made
Subject Knowledge	Demonstrates mastery of the topic in each of the following categories; what the disease is, how people get it, symptoms, diagnosis, treatment, and interesting facts.	Demonstrates accurate knowledge of the topic in each of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment	Demonstrates only some knowledge of the topic, and is missing one or two of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment	Demonstrates little knowledge of the topic, and is missing more than three of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment
Organization and Coherence	Organizes information coherently, stays on the topic	Organizes most information, stays on the topic	Generally organizes information, occasionally strays from the topic	Poorly organizes information, often strays from the topic
Physical Presentation	Always speaks clearly/loudly, actively engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Usually speaks clearly/loudly, usually engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Speaks clearly/loudly, occasionally engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Does not speak clearly/loudly, neglects to engage the audience, rarely makes and maintains eye contact or uses movement to focus attention/interest
Language Convention	Uses appropriate grammar and vocabulary	Mostly uses appropriate grammar and vocabulary	Makes some errors in grammar and vocabulary	Makes many mistakes in grammar and vocabulary
Visual Aids	Creatively uses a variety of effective visual aids and/or other methods of delivery	Uses visual aids moderately effectively and/or other methods of delivery	Moderately ineffective use of some visual aids and/or other methods of delivery	Does not use of visual aids and/or other methods of delivery
Scholarly Sources	More than two additional sources included and appropriately cited	Two additional scholarly sources included and appropriately cited	Either only one additional scholarly source included or not appropriately cited	Lacking two additional scholarly sources and not appropriately cited

Appendix E

Biotechnology Unit Plan Student's Manual (begins next page)

Grand Valley State University and Kent Intermediate School District Early College Program's

Biotechnology Unit Plan

Student's Manual

Developed by Meagan Treadway

In Collaboration with

Dr. Deborah Herrington - GVSU

Russell Wallsteadt - KISD

Owner of this Manual

(Student Name)

_____

Table of Contents

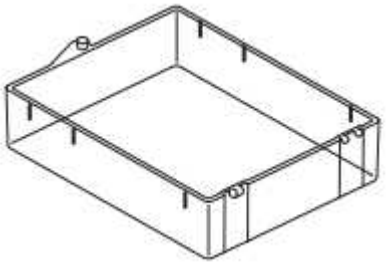
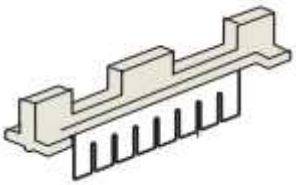
Lesson 1 Electrophoresis & Genetic Trait Exploration

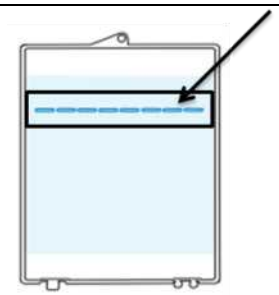
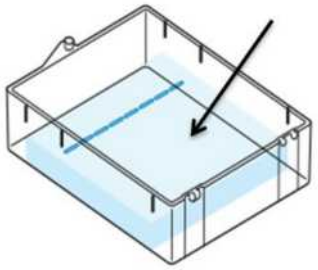
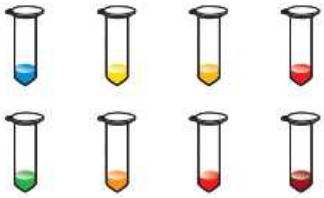
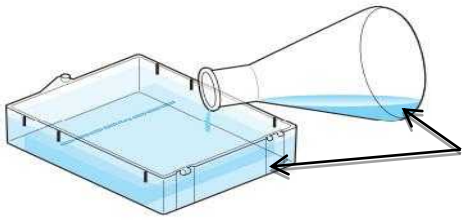
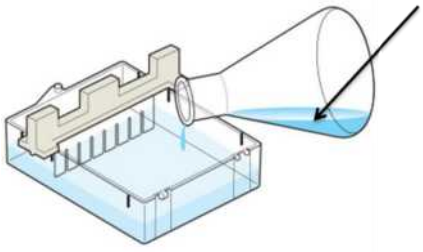
Identifying the Components

Agarose gel electrophoresis is a method that uses electrical current to separate macromolecules, such as DNA, RNA, and proteins, and their fragments based on their size and charge. Before you can use this technique, though, you need to know what materials you'll be working with during the lab. Take a look at the components, both materials and equipment, in front of you on your lab bench and at the shared table for the lab. Is there anything you recognize right away? What are some things you have never seen before? Can you work with your lab mates to identify the different components and take a guess at what they might be used for during agarose gel electrophoresis? Below are some helpful pictures and names for the different components you might encounter. See if you can match any of them with one another.

Word Bank		
Wells	**Solidified Agarose Gel**	**Leads**
Buffer	**Pipet Tip**	**Comb**
Microcentrifuge Tubes	**Molten Agarose Gel**	**Centrifuge**
Electrodes	**Samples (Known and Unknown)**	**Pipet**
Power Supply		**Gel Electrophoresis Chamber**

Select words from the word bank above and fill in spaces A-N to identify the pictures below them.

A: _____	B: _____	C: _____
		

D: _____	E: _____	F: _____
		
G: _____	H: _____ I: _____	J: _____
		
K: _____	L: _____	
		
M: _____	N: _____	
		

Lesson 1 Electrophoresis & Genetic Trait Exploration

Determining Your Procedure

Now that you are familiar with the different components with which you will be working, how should you move forward with your agarose gel electrophoresis procedure? What are some things you should consider related to your sample, putting together the electrophoresis apparatus, and making and preparing your gel for samples? How long do you think the gel should be run and how will you know when it is done? What might you need to do with the gel once the samples are done running? Using the space below and on the back of this page, work with your lab mates to number and describe several steps, in logical order, you would use to complete this procedure.

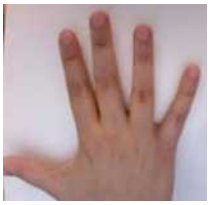
Lesson 1 Electrophoresis & Genetic Trait Exploration

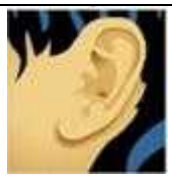
Human Genetic Traits Inventory

(Adapted from Biology Department, Grand Valley State University)

In simple Mendelian inheritance, traits are controlled by a single gene where one allele is received from each parent. If an allele is dominant, the trait will be expressed even if only one copy is inherited from the parents. If an allele is recessive, two copies must be inherited for the trait to be expressed. Those individual who inherit two matching alleles are called homozygous while those who inherit two non-matching alleles are termed heterozygous. The genotype is the total representation of a person's genes, and the phenotype is the expression of those genes. Take a look at the following example for tongue rolling.

The ability to *roll your tongue* upward is a dominant phenotype which can be represented by the genotypes RR (homozygous dominant) and Rr (heterozygous). The inability to roll your tongue would be the recessive phenotype, represented by the genotype rr (homozygous recessive).

Trait	Dominant Form	Recessive Form
1. Tongue Rolling *(Image source: drgcdms.blogspot.com)*	 RR or Rr	 rr
2. Facial Dimples *(Image source: reachoutmichigan.org)*	 DD or Dd	 dd
3. Bent Little Finger *(Image source: carolguze.com)*	 BB or Bb	 bb

4. Free Ear Lobes *(Image source: drgcdms.blogspot.com)*	 FF or Ff	 ff
5. Mid-digital Hair *(Image source: drgcdms.blogspot.com)*	 HH or Hh	 hh
6. Hand Clasping *(Image source: learn.genetics.utah.edu)*	 LL or Ll	 ll
7. Widows Peak *(Image source: drgcdms.blogspot.com)*	 WW or Ww	 ww
8. Hitchhiker's Thumb *(Image source: wikispaces.psu.edu)*	 TT or Tt	 tt
9. Cleft Chin *(Image Source: genetics.thetech.org)*	 CC or Cc	 cc
10. Eye Color *(Image source: bbc.co.uk)*	 MM or Mm	 mm

Human Genetic Traits Inventory

After reviewing the phenotypes on the previous page, complete the inventory below by determining your own phenotype and genotype as best you can. You may need to think about the traits in your parents to help determine the details. We will then take a look at the total inventory for our class when everyone is complete.

Trait	Symbol	Dominant Phenotype	Your Phenotype	Your Genotype	Number of Phenotypes in Class	
					Dominant	Recessive
1. Tongue Rolling	R, r	Rolled				
2. Facial Dimples	D, d	Present				
3. Bent Little Finger	B, b	Bent				
4. Free Ear Lobes	F, f	Free				
5. Mid-digital Hair	H, h	Present				
6. Hand Clasping	L, l	Top Left Thumb				
7. Widow's Peak	W, w	Present				
8. Hitchhiker's Thumb	T, t	Absent				
9. Cleft Chin	C, c	Present				
10. Eye Color	M, m	Melanin (Brown)				

Lesson 1 Electrophoresis & Genetic Trait Exploration

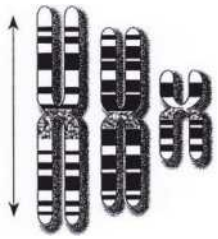
Human Karyotype Exercise

(Adapted from Biozone International 1995-2003)

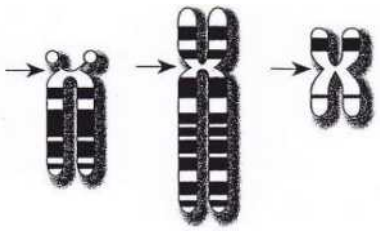
Normal human cells contain 23 pairs of chromosomes, one chromosome of each pair coming from each parent. However, during the production of gametes, occasionally genetic material is lost or rearranged. Many times these changes are so severe that either fertilization does not occur or the resulting embryo will not survive. However, approximately one in 156 live births has some kind of chromosomal abnormality.

An example of a chromosome abnormality is Down Syndrome in which there is an extra chromosome 21. This occurs in 1 in 700 births in the U.S. and this condition is associated with mental retardation, characteristic facial features, heart defects, respiratory infections, leukemia, and Alzheimer's disease. For these cases, a karyotype may offer information about abnormalities related to chromosome structure and number, in addition to the sex of the fetus.

Karyotypes are created by taking a sample of blood, or uterine fluid for fetuses, and staining and photographing the chromosomes from a cell. A cell undergoing mitosis is required, preferably in metaphase, so that the chromosomes are condensed and visible under a microscope just after replication. The photograph is enlarged and cut up into individual chromosomes which are then arranged according to homologous pairs and ordered by size (placing the sex chromosomes last; the X being the larger, and Y being the smaller of the sex chromosomes). Homologous pairs can be identified based on chromosome length, centromere position, banding pattern, and the presence of satellite endings.



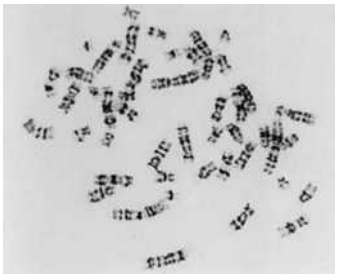
Chromosome Length



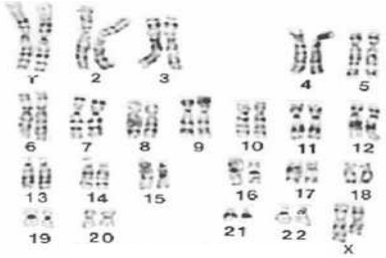
Centromere Position

Banding Pattern

Satellite Endings



Spread of Human Chromosomes
(Source: Foundation for Research in Genetics & Endocrinology)



Karyotype of Human Chromosomes
(Source: Brazilian Journal of Genetics, Vol. 20, No. 3, 1997)

For this exercise, you will be working with a partner to create your own karyotype. From the chromosome spread on the next sheet, carefully cut out each chromosome. Find the homologous pairs by matching length, the position of the centromere, and the banding pattern. Arrange the pairs on the karyotype form below in order from longest to shortest, placing the sex chromosomes at the end. Tape lightly at first. Once you are confident that you have them placed correctly, tape them down more securely. Can you answer the following questions about your sample?

g) What is the sex of this individual: Male or Female ? (circle one)

h) Determine the state of the chromosomal arrangement: Normal or Abnormal ? (circle one)

i) If the arrangement is abnormal, state in what way: _____

1	2	3
A		

4	5
B	

6	7	8	9	10	11	12
C						

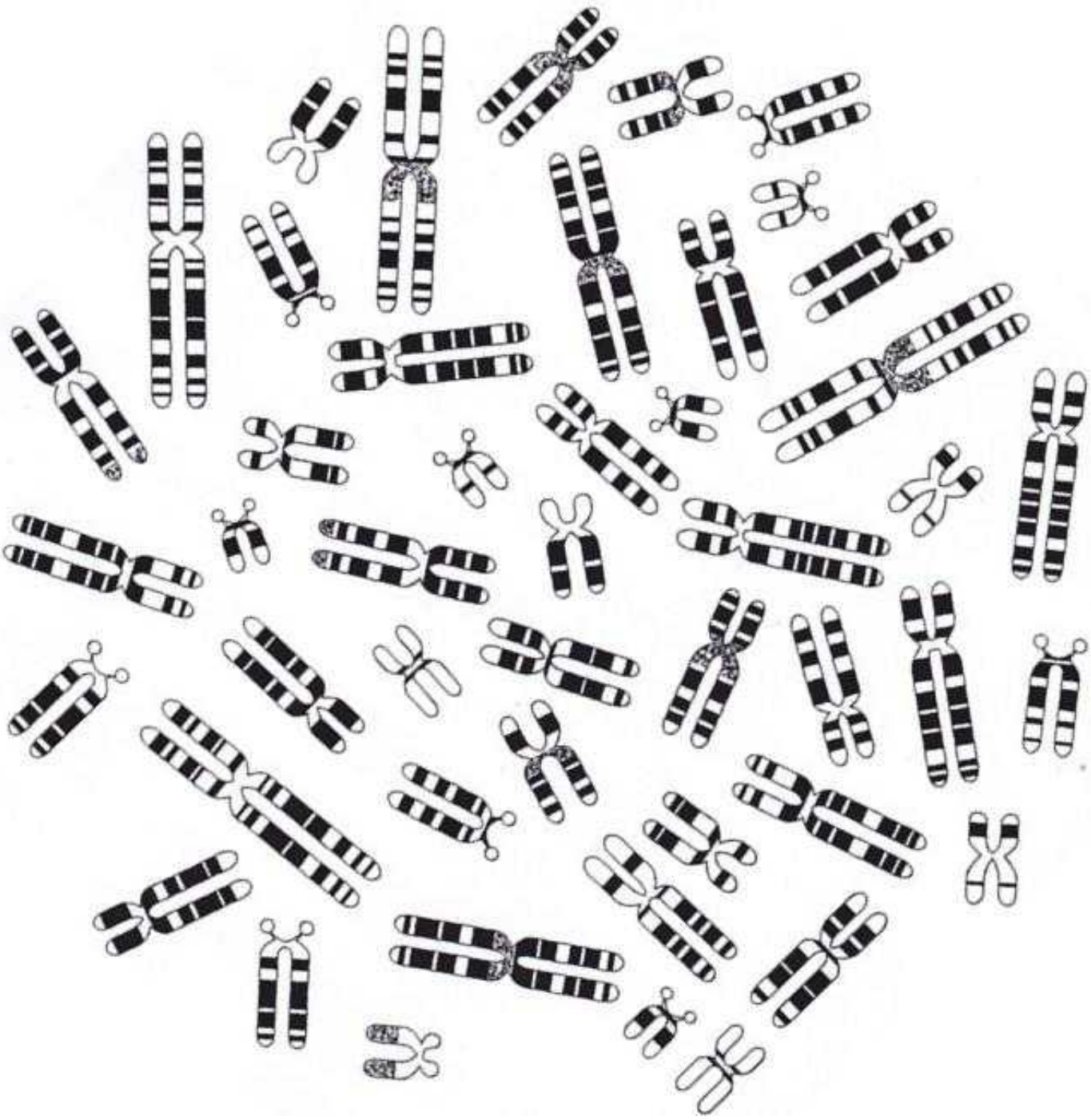
13	14	15
D		

16	17	18
E		

19	20
F	

21	22
G	

X	X or Y
Sex	



Lesson 2 Dye Electrophoresis & Gene Exercises

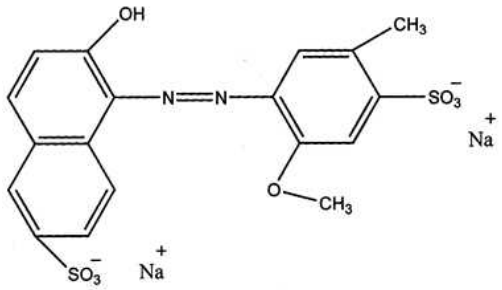
Dye Agarose Electrophoresis Procedure

(Adapted from Biotechnology Explorer, STEM Electrophoresis Kit, 166-5080EDU, Bio-Rad)

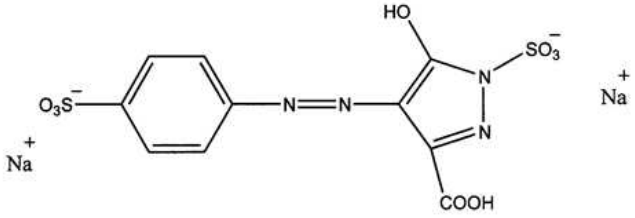
Reagents (provided by kit)	**Equipment & Supplies (provided by kit)**	**Shared Items (not provided by kit)**
Dye extraction solution, 25 ml 1 bottle	2 ml microcentrifuge tubes, 72 tubes	9 V batteries, 6–10
Blue 1 reference dye, 150 µl, 1 vial	Hinged plastic boxes, 1 box	Plastic rulers or plastic card to cut gels, 2
Yellow 5 reference dye, 150 µl, 1 vial	Paper clips, 4	2–20 µl adjustable-volume micropipette or 10 µl fixed-volume micropipette, 2
Yellow 6 reference dye, 150 µl, 1 vial	Black alligator clip leads, 2	2–200 µl pipet tips, 1,000/bag, 1 bag
Red 40 reference dye, 150 µl, 1 vial	Red alligator clips leads, 2	Eyedroppers or 100–1,000 µl adjustable-volume micropipette, 2; or disposable plastic transfer pipettes (DPTPs), 1 box; or 100–1,000 µl pipet tips, 1 bag
Electrophoresis buffer, 50x TAE, 100 ml, 1 bottle	8-well combs, 2	Marking pen, 2
Molecular biology grade agarose, 5 g, 1 bottle		Plastic cups or small beakers, 8
		Microwave oven or hot plate, 1
		Balance, 1
		Distilled water, 1 liter
		500 ml Erlenmeyer flask for microwaving agarose, 1
		Candies with a variety of color coatings variable (M&M's, Skittles, or Kool-Aid drink mixes)
		Optional
		Microcentrifuge or mini centrifuge, 1
		Digital camera for imaging gels, 1
		Microcentrifuge tube racks, 8

Structure of Reference Dyes

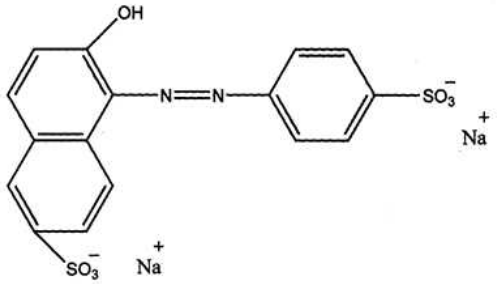
The Structure of Red 40



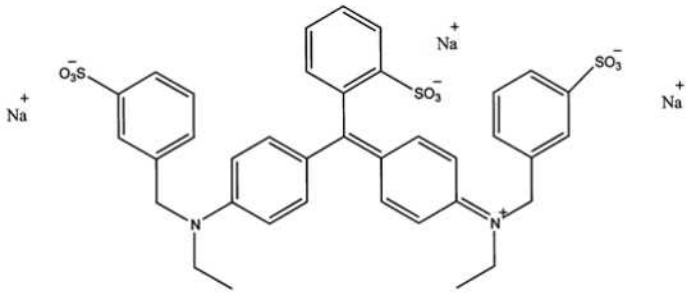
The Structure of Yellow 5



The Structure of Yellow 6



The Structure of Blue 1

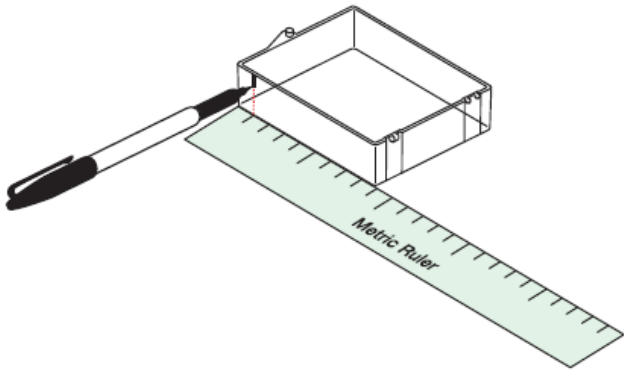


Unknown Sample #: _____

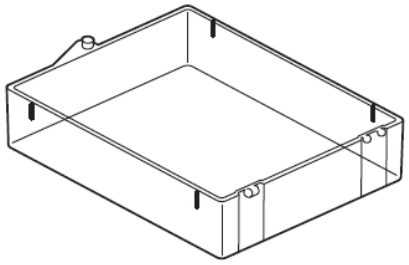
Hypothesis: _____

Part I: Prepare the gel boxes

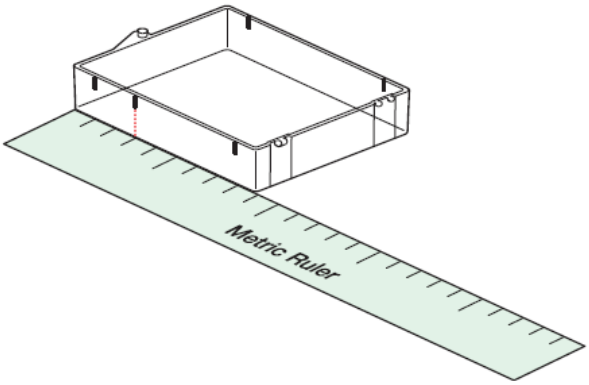
1. Measure a distance 1 cm from the end of the box on the longest side, and with a marking pen, and make a dash on the outside of the box.



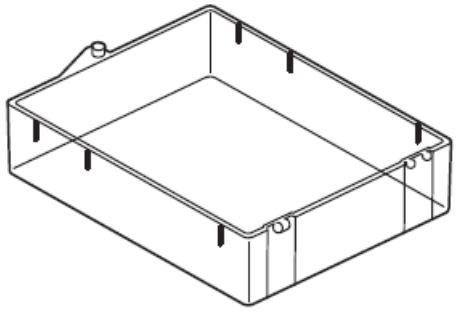
2. Repeat step 2 so that you have a mark 1 cm from the end of each of the longest sides of the box.



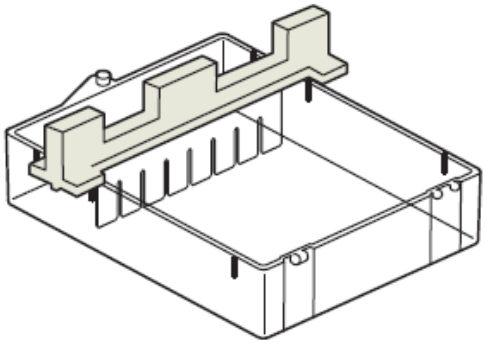
3. Measure a distance 3 cm from the end of the box on the longest side, and with a marking pen, and make a dash on the outside of the box.



4. Repeat step 4 on the opposite side of the box. When you have finished, each side of the box should have three marks.

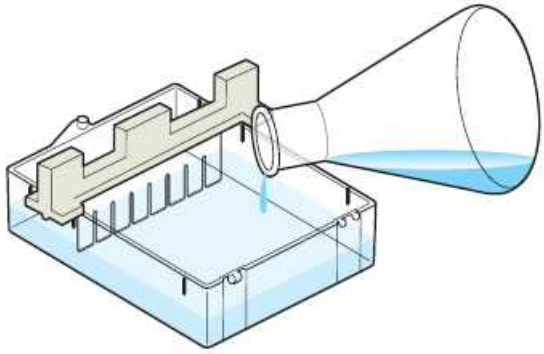


5. Place your 8-well comb on the marks that are 3 cm from the end. Make sure that the comb is centered so that none of the clear plastic well-formers touch the plastic box and that the comb is straight across the box.

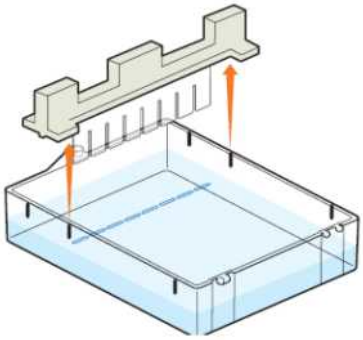


6. Carefully pour 50 ml of molten agarose into the box and allow the gel to solidify for 10–20 min. The gel will appear cloudy, or opaque, when ready to use.

   CAUTION: Always were protective gloves, goggles, and lab coat while preparing and casting agarose gels. Molten agarose or the flasks containing hot agarose can cause severe burns if allowed to contact skin.

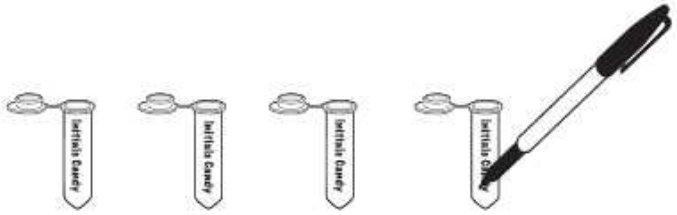


7. Carefully remove the comb from the solidified gel by pulling gently in an upward direction.



Part II: Dye extraction from candies (this has already been completed for you)

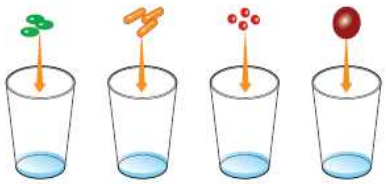
1. Label the four microcentrifuge tubes with the names and colors of the candies you are using.



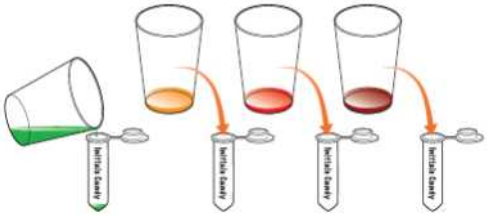
2. Label four cups with the names and colors of the candies you are using.

3. Using an eyedropper or pipet add 0.5 ml of dye extraction solution to each cup. Use the volume marks on the 2 ml microcentrifuge tube to measure the correct volume.

4. Place your candy into the appropriately labeled cup and swirl the candy in the dye extraction solution. If using a candy such as M&M'S or Skittles, just dissolve the color coating off until you get to the white layer of the candy. For all other candies, try to get as dark a solution of dye as possible.



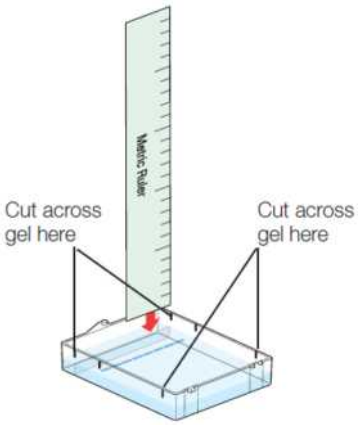
5. Remove your candy from the cup. Pour the solution containing the dissolved colored candy coating into the appropriately labeled microcentrifuge tube.



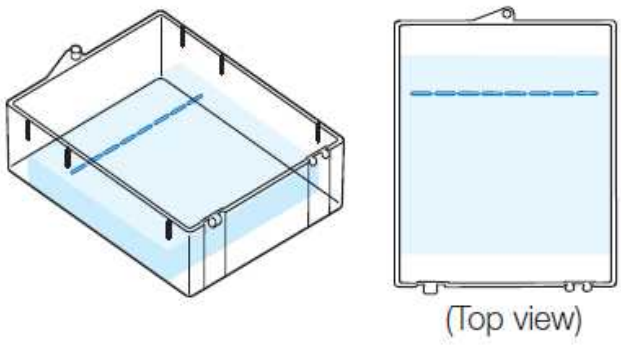
Part III: Agarose Gel Electrophoresis

1. Obtain your agarose gel in the plastic chamber. If you stored your gel after preparing it, pour off the 25 ml of 1x TAE buffer.

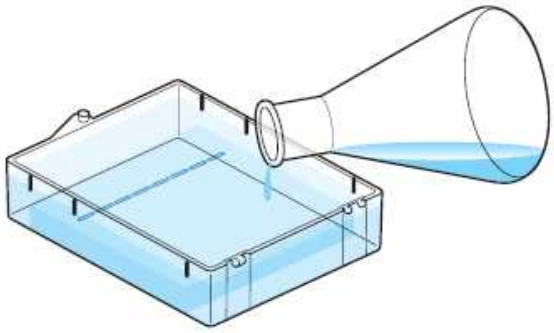
2. Using your ruler and following the marks you made one centimeter from the end of the box, cut a slab off the end of the gel using the end of a ruler. Press straight down through the gel to the box — do not slice across the gel. Loosen the slab by sliding the ruler between the end of the gel and the box end, then lift out the slab and discard.



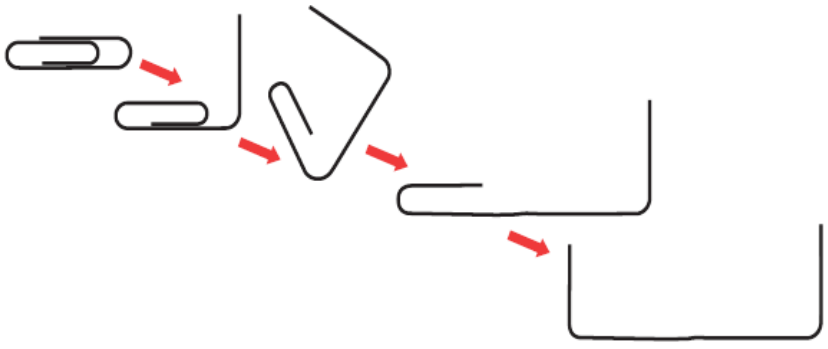
3. Repeat at the other end of the gel.



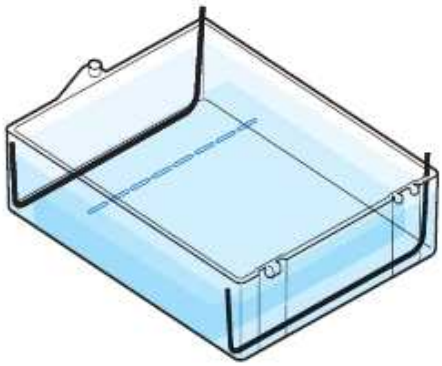
4. Add 55 ml of 1x TAE buffer to the box.



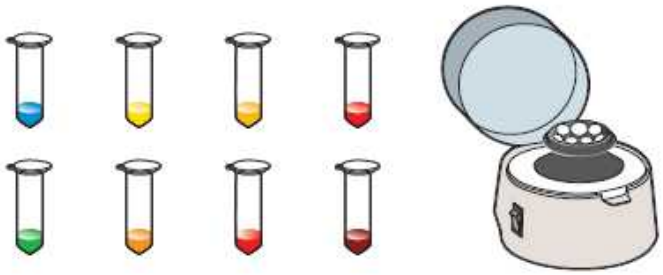
5. Construct your electrodes from two paper clips. Carefully straighten the paper clip and bend the two ends so they are perpendicular to the rest of the clip. Place your completed electrode on a flat surface. If it does not lie flat (in other words, if one of the angled pieces is not in the same plane as the rest of the electrode), hold the two ends and twist gently until the electrode will lie flat. The longer end will stick above the gel box – this is where you will attach the alligator clip.



6. Place the electrodes into the gel box with the long ends on the same side. The electrodes should be as close to the end of the box as possible (as far away from the gel as possible).



7. Prepare your extracted candy dye samples and reference dyes. If a centrifuge is available, pulse spin the microcentrifuge tubes in the centrifuge to bring all the liquid to the bottom of the tube and to settle any insoluble particles. Spin down your dye standard samples as well, if needed.



8. Using a separate tip for each sample, load 10 µl of each sample into 8 wells of the gel in the following order:

Lane 1: Blue 1 reference dye

Lane 2: Yellow 5 reference dye

Lane 3: Yellow 6 reference dye

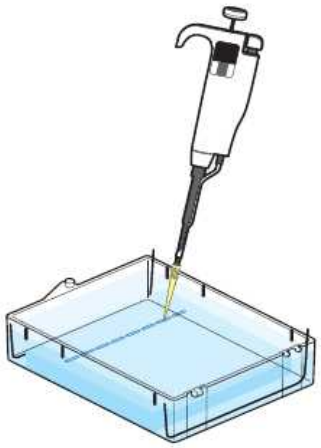
Lane 4: Red 40 reference dye

Lane 5: Candy 1 dye extract

Lane 6: Candy 2 dye extract

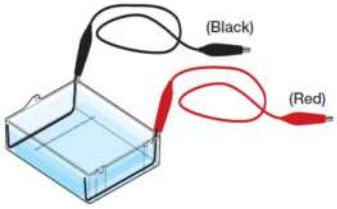
Lane 7: Candy 3 dye extract

Lane 8: Candy 4 dye extract

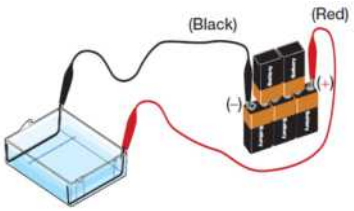


9. Assemble your battery tower by connecting negative nodes to positive nodes.

10. Attach the black alligator clip to the long end of the paper clip and box at the end of the box closest to the sample wells. Make sure the paper clip still remains on the bottom of the gel box under the buffer. Repeat the process for the red alligator clip and the electrode at the other end of the box.

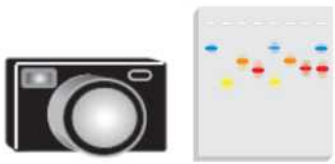


11. When you are ready to begin your electrophoresis run, attach the free black alligator clip on your lead to the (–) terminal of your battery tower and the free red alligator clip on your lead to the (+) of your battery tower. You should notice bubbles coming off of the paper clip electrodes if the circuit is complete.



12. Allow your gel to run for 20 min. Disconnect the red and black alligator clips from the battery tower.

13. Optional: Take a photograph of the gel for your records.



Unknown Sample #: _____

Results: _____

Lesson 2 Dye Electrophoresis & Gene Exercises

Homework: Genetic Traits Video Questions

(Adapted from 23andMe.com)

- Visit the following website: www.23andMe.com/gen101/
- Watch the following videos and answer the questions below: What Are Genes? What are SNPs? Where Do Your Genes Come From? What Are Phenotypes?

What Are Genes?

1. Where do most of the genes reside in your body? _____
2. "DNA is a double-stranded molecule composed of _____, _____, and four different bases_____, _____, _____, and _____."
3. "Most genes are recipes for making specific _____."
4. "Gene _____ turn different genes on and off in different cells to control cell function."
5. How many pairs of chromosomes do humans have? _____
6. What percentage of the DNA in your chromosomes do you share with chimpanzees? _____ . Other humans? _____

What Are SNPs?

1. An entire set of 23 human chromosomes is called a _____.
2. Variation at a single base pair is called a SNP, or _____ _____ _____.
3. An SNP is created when a single base pair is _____, _____, or _____.
4. True or False. SNPs account for the genetic variation between you and other humans.

5. True or False. Many SNPs lead to no observable differences between people at all.

6. True or False. SNPs can be used to trace ancestry.

Where do Your Genes come from?

1. You inherit one _____ of chromosomes from each _____, which together are called _____ chromosomes.

2. Most cells contain two sets of chromosomes, but _____ and _____ cells each contain only one set of chromosomes.

3. Your siblings also receive one set of genes from each of your parents, but not necessarily the same combination of genes unless you are _____ _____.

4. When homologous chromosomes exchange DNA by crossing over, this is called genetic _____.

5. Ancestry along your father's line is easier to trace through the _____, and through your mother's line it is easier to trace through the _____.

What Are Phenotypes?

1. Your _____ _____ traits, also called your phenotype, results from the interaction between your genes and the environment.

2. Give two examples of phenotypes which are controlled by the interaction between your genes and the environment, such as diet and exercise; 1) _____and 2) _____.

3. True or False. Not much is known about how your genes affect your personality.

Lesson 3 Class Discussion of Electrophoresis and Genetic Traits

Review of DNA and Genes

(Adapted from Genetic Science Learning Center and Bio-Rad Biotechnology Explorer

Image Source: www.bio-rad.com)

Review Questions

1. What is DNA?

2. What does "DNA" stand for?

3. What is the four-letter DNA alphabet and what are the special rules by which the alphabet pieces bond together?

4. What is a gene?

5. What are genes made of?

6. For what molecule do genes contain the instructions for building?

7. What is a chromosome?

8. How many chromosomes does a human cell hold?

9. How are the human sex chromosomes labeled?

10. What provides the "blueprint" for making a protein?

11. What is heredity?

12. Why aren't children identical to either one of their parents?

13. In humans, how many chromosomes does each parent pass on to their offspring?

14. Does the second child in a family inherit the exact same chromosomes as the first? Do both babies have a complete set?

15. What is a trait?

16. Give an example of how an environmental factor can influence a trait.

17. Briefly explain how the Hitchhiker's Thumb trait is determined using the following words: allele, dominant, recessive, homozygous, heterozygous. You may draw pictures if you wish.

Lesson 3 Class Discussion of Electrophoresis and Genetic Traits

Introduction to Electrophoresis

(Adapted from Biotechnology Explorer, STEM Electrophoresis Kit, 166-5080EDU, Bio-Rad)

1. When you analyzed the results of your gel, did any of your experimental samples contain dyes that did not match the four reference dyes? For example, did any of your samples produce:

   a. Dyes that are a different size than any of the standard bands?

   b. Dyes that are a different color than any of the standard bands?

   c. More than one color band?

   d. Dyes that moved a different distance compared to the standard bands?

2. We powered our electrophoresis with 9 V batteries connected in a series (multiple batteries with negative electrodes connected to positive electrodes to form a chain). For batteries connected in series, the total voltage is equal to the sum of the voltages of the individual batteries.

   a. How many batteries did we use? _____

   b. What would be the voltage if we used three batteries in series? _____ volts

   c. What if we used five batteries? _____ volts

   d. Would we expect our samples to migrate faster through the gel if we used three batteries or five batteries? Why? What might be some disadvantages of using a higher voltage for the power supply?

3. For these experiments, we used a 1% agarose gel to separate the dyes. How do you think the results would differ if we used a 3% agarose gel? A 0.8% agarose gel?

4. What are some properties that need to be considered when picking a material to construct an electrophoresis box?

5. In this kit, you used zinc-coated steel paper clips to construct electrodes. Commercially-made gel boxes use platinum wire for electrodes. What are the characteristics needed for a good electrode?

6. Which direction would you expect a dye to run if it has no charge?

7. Calculate the rate that each dye moves in the gel.

    a. Using the photograph of your gel, measure the distance each reference dye moved from the wells in cm

    b. Divide the distance traveled by each reference dye by the time that you ran your gel (in minutes)

    c. Which dye has the fastest rate of movement in cm/min?

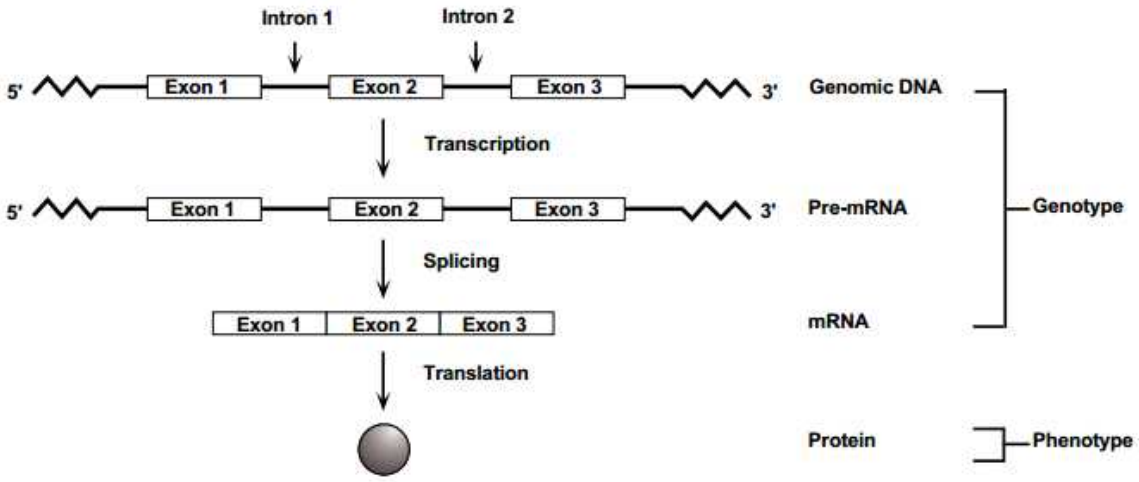
    d. Why might the distance travelled differ between the dyes?

DNA Processing

As we just reviewed, each human carries 23 pairs or 46 single chromosomes (23 from the mother and 23 from the father). It is estimated that these 46 chromosomes contain approximately 30,000 – 50,000 genes. Each chromosome contains a series of specific genes, dependent on its size. Each of the homologous chromosome pairs contains similar genes.

Each gene holds the code for a particular protein. Interestingly, the 30,000 – 50,000 genes only comprise 5% of the total chromosomal DNA. The other 95% is noncoding DNA. This noncoding DNA is found not only between, but within genes, splitting them into segments. The exact function of the noncoding DNA is not known, although it is thought that noncoding DNA allows for the accumulation of mutations and variations within genomes.

When RNA is first transcribed from DNA, it contains both coding and noncoding sequences. While the RNA is still in the nucleus, the noncoding **introns** (**in = stay within the nucleus**) are removed from the RNA while the **exons** (**ex = exit the nucleus**) are spliced together to form the complete messenger RNA coding sequence for the protein (see image below). This process is called RNA splicing and is carried out by specialized enzymes called spliceosomes.



Introns often vary in size and sequence among individuals, while exons do not. This variation is thought to be the result of the differential accumulation of mutations in DNA throughout evolution. These mutations in our noncoding DNA are silently passed on to our descendants; we do not notice them because they do not affect our phenotypes. However, these differences in our DNA represent the molecular basis of DNA fingerprinting used in human identification and studies in population genetics.

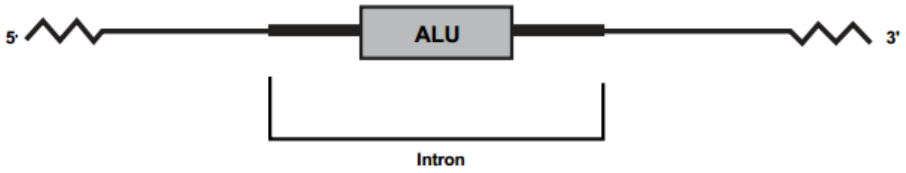
Lesson 4 Introduction to Human *Alu* PV92 & Isolation of Cheek Cell DNA

What Is Alu PV92?

(Adapted from DNA Learning Center, Cold Spring Harbor Laboratory and Bio-Rad Biotechnology Explorer)

Background

The function of noncoding pieces of DNA is not well understood; however, upon closer investigation scientists noticed several unique genetic elements repeated in a number of different locations. One of the first repeating elements found in all primates is called *Alu*. *Alu* repeats are approximately 300 base pairs long and are sometimes referred to as a "jumping gene" as *Alu* can get copied from one place to another place in the DNA via reverse transcriptase. It is believed that *Alu* first emerged a very long time ago, as many as 60 million years ago, as it now exists in all primates and is inserted in different locations across the genome of each species.



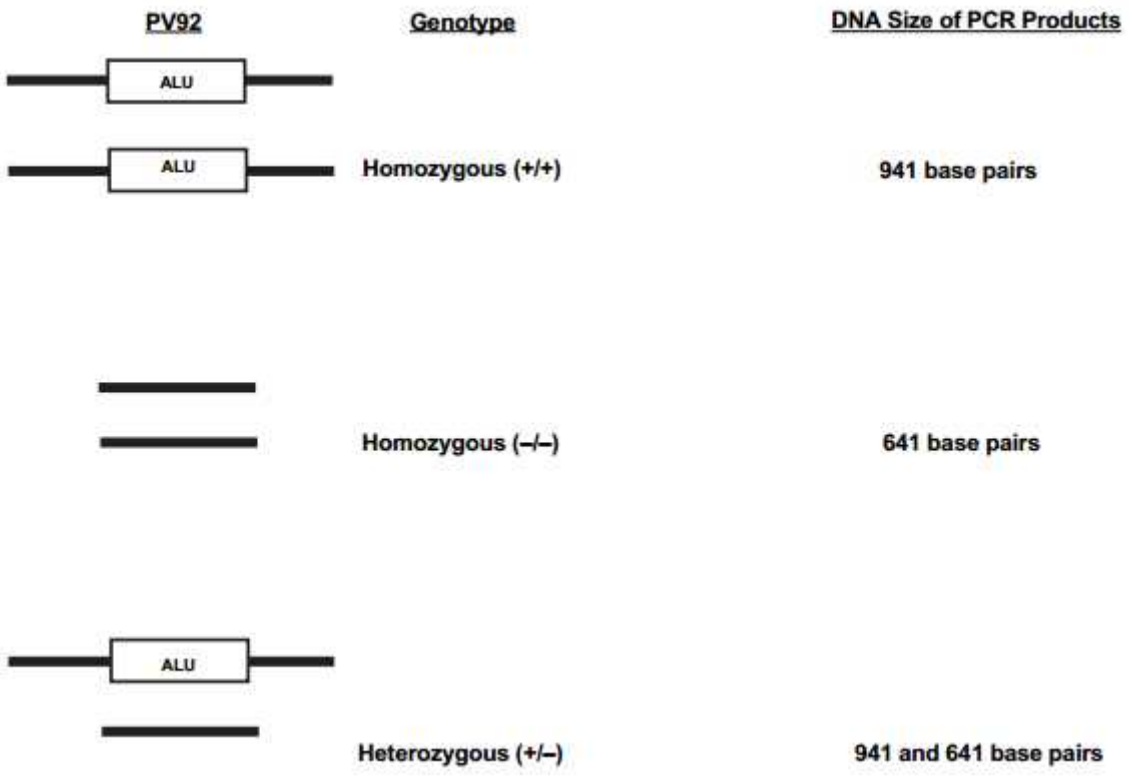
Most *Alu* insertions occur in non-coding regions of the DNA and as of yet have not been found to have any beneficial or adverse effects. However, some *Alu* insertions have been linked to certain conditions. For example, an *Alu* insertion in the NF-1 gene is responsible for neurofibromatosis I, and *Alu* insertions in introns of genes for tissue plasminogen activator (TPA) and angiotensin converter enzyme (ACE) are associated with heart disease.

Once an *Alu* inserts at a chromosome locus, it can copy itself for transposition, but there is no evidence that it is ever removed from a chromosome locus. So, each *Alu* insertion is stable through evolutionary time. Each is the "fossil" of a unique transposition event that occurred only once in primate evolution. Like genes, *Alu* insertions are inherited in a Mendelian fashion from parents to children. Thus, all primates showing an *Alu* insertion at a particular locus have inherited it from a common ancestor. This is called identity by descent.

Although many *Alu* insertions are found in all primate species, there are estimated 500-2000 insertions that are specific to the human genome. Many of the human-specific *Alu* insertions are "fixed" in the populations meaning that both of the paired chromosomes have an insertion at the same locus (position). However, a number of human-specific *Alu* insertions are dimorphic – an insertion may be present or absent on each of the paired chromosomes of different people. These dimorphic *Alu* insertions are the ones that have occurred most recently, within the last million years. These dimorphisms show differences in allele and genotype frequencies between modern populations and are tools for reconstructing human prehistory.

Current Investigation

Today we are going to look at *Alu* PV92 which is a human-specific *Alu* insertion on chromosome 16. It is dimorphic, meaning the element is present in some individuals and not others. The PV92 genetic system has only two alleles indicating the presence (+) or absence (-) of the *Alu* transposable element on each of the paired chromosomes. This results in three PV92 genotypes (++, +-, or --). The + and - alleles can be separated by size using gel electrophoresis.



Question

During this lab we are going to investigate the question; what is my *Alu* PV92 genotype?

Tasks:

5. Isolate DNA from our cheek cells.
6. Amplify DNA with PCR.
7. Analyze DNA using gel electrophoresis.
8. Report findings.

Lesson 4 Introduction to Human *Alu* PV92 & Isolation of Cheek Cell DNA

Isolation of Cheek Cell DNA Procedure

(Adapted from Bio-Rad Biotechnology Explorer Image Source: www.bio-rad.com)

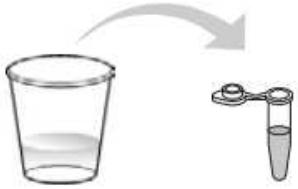
Reagents	Equipment & Supplies (provided by kit)	Shared Items (not provided by kit)
Saline Solution (0.9% NaCl), 10 ml	Foam micro test tube holders	P-20 micropipet
InstaGene matrix, 20 ml	Screw cap tubes	Pipet tips (filter type), 2-20 µl
	1.5 ml Micro test tubes, with attached caps	Permanent marker
		Biological waste container
		P-1000 or P-200 micropipet
		Pipet tips (filter type), 100-1000 µl or 20-200 µl
		Water baths (56 and 100 °C)
		Microcentrifuge or mini centrifuge
		Vortexer

Procedure

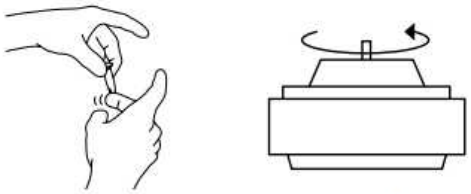
13. Each member of your team should have 1 screw cap tube containing 200 µl of InstaGene matrix, 1.5 ml micro test tube, and a cup containing 10 ml of 0.9% saline solution. Label one of each tube and a cup with your initials.

14. **<u>Do not throw away the saline solution after completing this step</u>**. Pour the saline from the cup into your mouth. Rinse vigorously for 30 seconds. Expel the saline back into the cup.

15. Set a P-1000 micropipet to 1,000 μl and transfer 1 ml of your oral rinse into the micro test tube with your initials. If no P-1000 is available, carefully pour ~ 1 ml of your swished saline into the micro test tube (use the markings on the side of the micro test tube to estimate 1 ml).



16. Spin your tube in a balanced centrifuge for 2 minutes at full speed. When the centrifuge has completely stopped, remove your tube. You should be able to see a pellet of whitish cells a t the bottom of the tube. Ideally, the pellet should be about the size of a match head. If you can't see your pellet, or your pellet is too small, pour off the saline supernatant, add more of your saline rinse, and spin again.



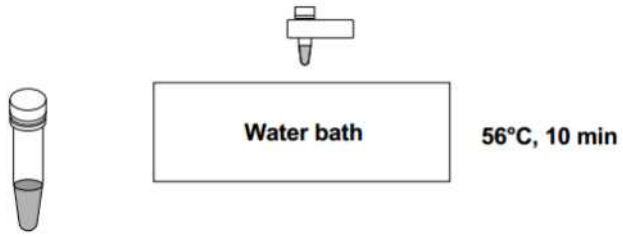
17. Pour off the supernatant and discard. Taking care not to lose your cell pellet, carefully blot your micro test tube on a tissue or paper towel. It's ok for a small amount of saline (~50 μl, about the same size as your pellet) to remain in the bottom of the tube.

18. Resuspend the pellet thoroughly by vortexing or flicking the tubes until no cell clumps remain.

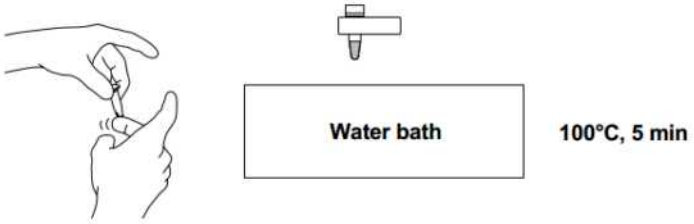
19. Using an adjustable volume micropipette set to 20 µl, transfer your resuspended cells into the screw cap tube containing the InstaGene with your initials. You may need to use the pipet a few times to transfer all of your cells.

20. Screw the caps tightly on the tubes. Shake or vortex the mix the contents.

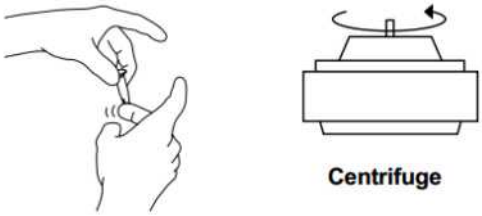
21. Place the tubes in the foam micro test-tube holder. When all members of your team have collected their samples, float the holder of tubes in a 56 °C water bath for 10 minutes. At the halfway point (5 minutes), shake or vortex your tubes several times. Place the tubes back in to the water bath for the remaining 5 minutes.



22. Remove the tubes from the water bath and shake them several times. Now float the holder with tubes in a 100 °C water bath for 5 minutes.



23. Remove the tubes from the 100 °C water bath and shake or vortex several times to resuspend the sample. Place the eight tubes in a balanced arrangement in a centrifuge. Pellet the matrix by spinning for 5 minutes at 6,000 x g (or 10 minutes at 2,000 x g).



24. Store your screw cap tube in the refrigerator until the next laboratory period.

Lesson 5 PCR Amplification of DNA

PCR Procedure

(Adapted from Bio-Rad Biotechnology Explorer Image Source: www.bio-rad.com)

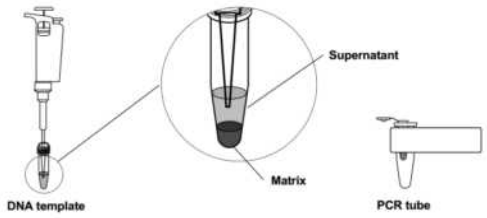
Reagents	**Equipment & Supplies (provided by kit)**	**Shared Items (not provided by kit)**
Human DNA sample	Foam micro test tube holders	P-20 micropipet
Complete master mix (with primers) on ice	PCR tubes	Pipet tips (filter type), 2-20 µl
	1.5 ml Micro test tubes, capless	Ice bucket with ice
		Permanent marker
		Biological waste container
		Microcentrifuge or mini centrifuge
		Thermal Cycler

Procedure

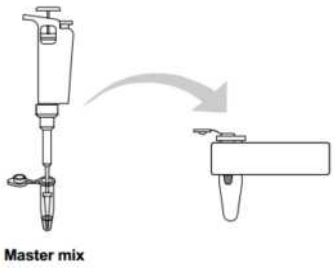
1. Obtain your screw cap tube that contains your DNA from your cheek cells from the refrigerator. Centrifuge your tubes for 2 minutes at 6,000 x g or for 5 minutes at 2,000 x g.

2. Each member of the team should obtain a PCR tube and capless micro test tube. Label each PCR tube on the side of the tube with your initials and place the PCR tube into the capless micro test tube as shown. Place the PCR tube in the foam micro test tube holder.

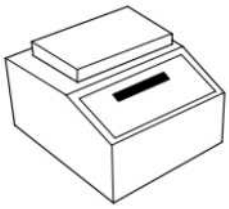
3. Transfer 20 µl of your DNA template from the supernatant in your screw cap tube into the bottom of the PCR tube. **Do not transfer any of the matrix beads into the PCR reaction because they will inhibit the PCR reaction.**



4. Locate the tube of yellow PCR master mix (labeled "Master") in your ice bucket. **Using a new pipette tip,** transfer 20 µl of the master mix into your PCR tube. Mix by pipetting up and down 2-3 times. Cap the PCR tube tightly and keep it on ice until instructed to proceed to the next step. Avoid bubbles, especially in the bottom of the tubes.



5. Remove your PCR tube from the capless micro test tube and place the tube in the thermal cycler.



6. When all of the PCR samples are in the thermal cycler, the teacher will begin the PCR reaction. The teacher will also include the electrophoresis controls in this reaction. The reaction will undergo 40 cycles of amplification, which will take approximately 3 hours.

Cycle	Step	Function	Temperature	Time
1	Step 1	Pre-denaturation	94°C	2 minutes
	Repeat 1 time			
2	Step 1	Denature	94°C	1 minute
	Step 2	Anneal	60°C	1 minute
	Step 3	Extend	72°C	2 minutes
	Repeat 40 times			
3	Step 1	Final Extension	72°C	10 minutes
	Repeat 1 time			
*	Step 1	Hold	4°C	Infinity

Lesson 5 PCR Amplification of DNA

Understanding PCR

(Adapted from Bio-Rad Biotechnology Explorer, www.dnalc.org, and University of Utah Health Sciences Image Source: www.bio-rad.com)

Introduction

Polymerase chain reaction (PCR) is a technique that was developed in 1983 by Kary Mullis, and is now universally used by scientists to quickly amplify segments of DNA. Amplification means to create millions of copies of specific regions of DNA without resorting to other labor intensive cloning procedures. Review the following videos to learn how PCR works:

http://www.dnalc.org/resources/animations/pcr.html

http://learn.genetics.utah.edu/content/labs/pcr/

The amplification takes place in a reaction mixture. This mixture must contain several key ingredients for the PCR to take place effectively. Do you know the purpose of each component in the reaction mixture? Fill in your answers below:

1. DNA template - _____.
2. Individual deoxynucleotides – _____.
3. DNA polymerase – _____.
4. Magnesium ions – _____.
5. Oligonucleotide primers – _____

   _____.
6. Salt buffer – _____

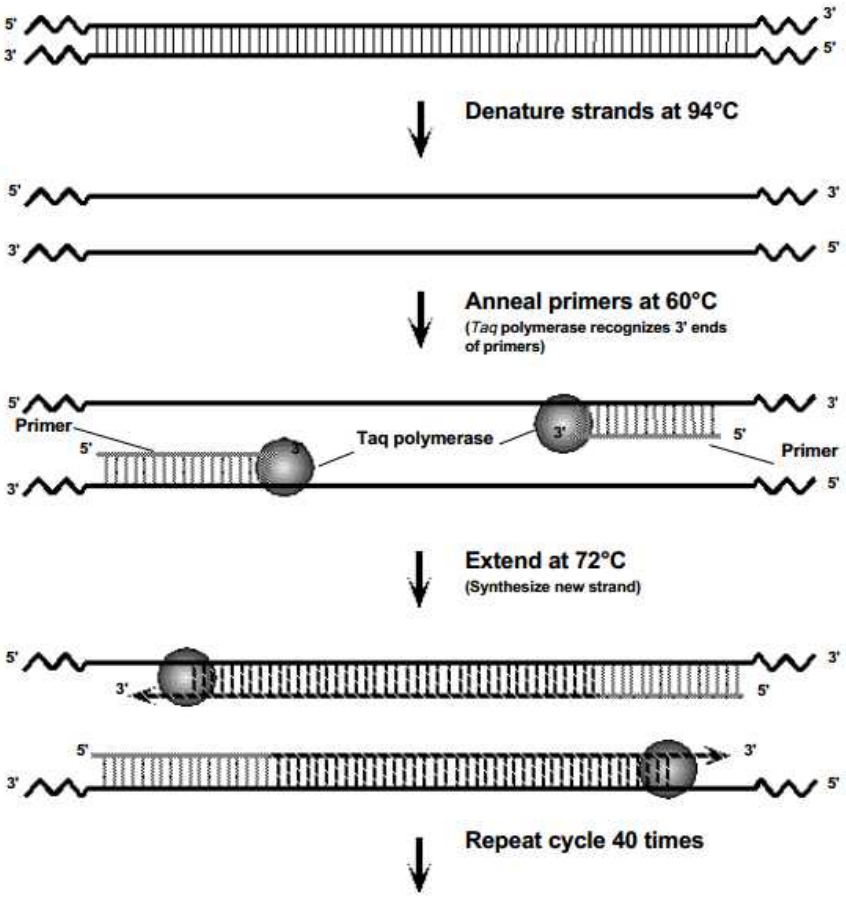
   _____.

If you recall, in this experiment, we are interested in making copies of the specific *Alu* PV92 region on chromosome 16. The template DNA is extracted from the cheek cell isolation you performed earlier in the lab. The primers for this reaction are as follows:

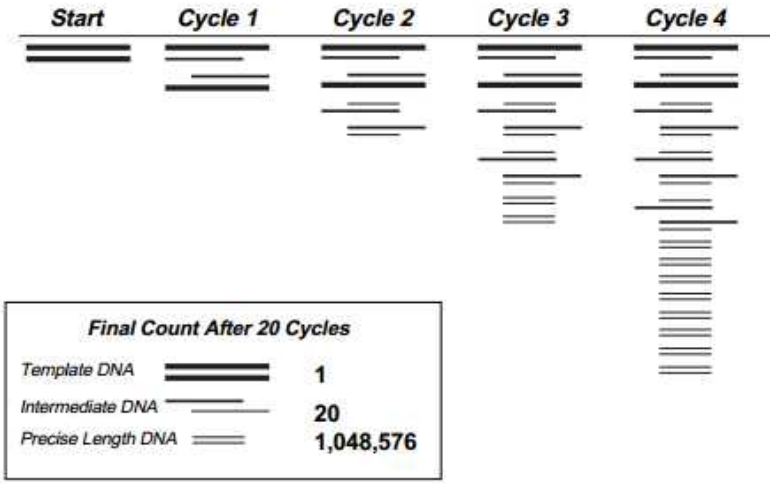
Forward primer: 5' GGATCTCAGGGTGGGTGGCAATGCT 3'

Reverse primer: 5' GAAAGGCAAGCTACCAGAAGCCCCAA 3'

The Reaction



After each of the 40 cycles of PCR, the DNA segment of interest will grow in copy number exponentially (X^n). Using this formula, how many copies would you have after 2 cycles; after 4 cycles?



Lesson 6 DNA Analysis by Gel Electrophoresis

DNA Electrophoresis Procedure

(Adapted from Bio-Rad Biotechnology Explorer Image Source: www.bio-rad.org)

Reagents	**Equipment & Supplies (provided by kit)**	**Shared Items (not provided by kit)**
Prepared PCR samples	Foam micro test tube holders	P-20 micropipet
PV92 XC DNA loading dye	Gel staining tray	Pipet tips (filter type), 2-20 µl
MMR (DNA standards)		Permanent marker
Fast Blast DNA stain, 1x or 100x solution		Gel box and power supply
1x TAE electrophoresis buffer		Biological waste container
Amplified positive control samples		Microcentrifuge or mini centrifuge
PV92 homozygous (+/+)		**For Quick Staining Protocol**
PV92 homozygous (-/-)		Warm tap water for detaining
PV92 heterozygous (+/-)		Large containers for destaining
Molten agarose, 50 ml		**Optional**
		Gel support film
		Clear acetate sheets for tracing gels
		Rocking platform

Part I: Prepare The Agarose Gels

The recommended gel concentration for this exercise is 1%, and the molten agarose gel has already been prepared to this concentration by adding 1 g of agarose to 100 ml of 1x TAE electrophoresis buffer. To make the electrophoresis gels, complete the following procedure:

1. Be sure the gel molding tray is constructed following your instructor's directions. Also ensure that it is setting on a level surface.

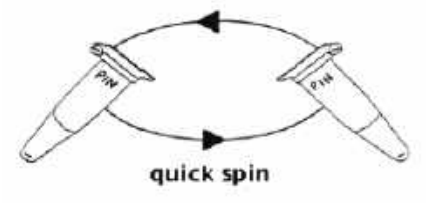
2. In an Erlenmeyer flask, obtain 50 mL of the prepared agarose gel. Slowly pour the agarose into the molding tray without creating bubbles. If bubbles do occur, you can use the tip of a plastic pipet tip to clear the bubbles off to the edge of the gel.

3. Once all the agarose is poured and bubbles are cleared, place the comb into the gel and allow it to cool.

4. The gel will become slightly cloudy once it has reached room temperature. At this point, the comb can be removed by gently moving it back and forth to loosen it from the gel. Pull it out slowly so as to not cause tears in your wells.

Part II: Gel Electrophoresis of Amplified PCR Samples

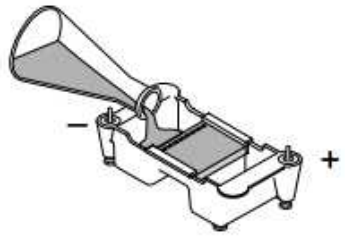
1. Remove your PCR samples from the refrigerator and place in the micro test tube holder. If a centrifuge is available, place the PCR tubes in the capless micro test tubes and pulse-spin the tubes (~ 3 seconds at 2,000 x g) to bring the condensation that formed on the lids to the bottom of the tubes.



2. Add 10 μl of PV92 XC loading dye to each PCR tube and mix gently.

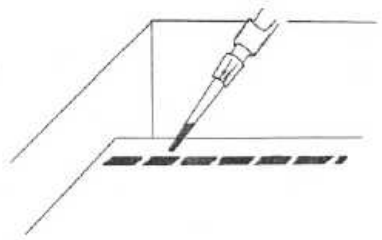
3. Ensure that your casting gel with the solidified gel in it is on the platform in the gel box. The wells should be at the cathode (-) end of the box, where the black lead is connected. If you haven't already, very carefully remove the comb from the gel by pulling straight up, slowly.

4. Pour ~ 250 ml of electrophoresis buffer into the electrophoresis chamber, until it just covers the wells.

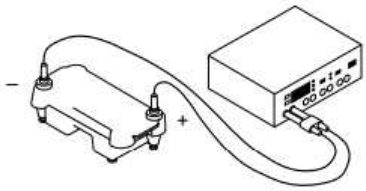


5. **<u>Using a clean tip for each sample</u>**, load the samples into the 8 wells of the gel in the following order:

Lane	Sample	Load Volume
1	MMR (DNA standard)	10 µl
2	Homozygous (+/+) control	10 µl
3	Homozygous (-/-) control	10 µl
4	Heterozygous (+/-) control	10 µl
5	Student 1:	20 µl
6	Student 2:	20 µl
7	Student 3:	20 µl
8	Student 4:	20 µl



6. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect the electrical leads to the power supply.



7. Turn on the power supply. Set it to 100 V and electrophorese the samples for 30 minutes.

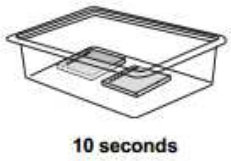
8. When electrophoresis is complete, turn off the power and remove the lid from the gel box.

Part III: Staining Your Gel

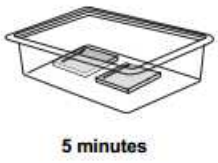
Warning: Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes.

6. *Stain Gels.* Carefully remove the gel tray and the gel from the gel box. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray. Pour approximately 120 ml of 100x stain into the staining tray. If necessary, add more 100x stain to completely submerge the gels. Stain the gels for 2 – 3 minutes, but not for more than 3 minutes. Using a funnel, pour the 100 x stain into a storage bottle and save it for future use. The stain can be reused at least 7 times.

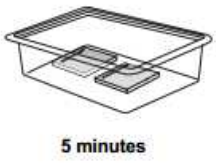
7. *Rinse Gels.* Transfer the gels into a large container containing 500 – 700 ml of clean, warm (40-55 °C) tap water. Gently shake the gels in the water for ~ 10 seconds to rinse.



8. *Wash Gels.* Transfer the gels into a second large container with 500 – 700 ml of clean, warm tap water. Gently rock or shake the gels on a rocking platform for 5 minutes. If no rocking platform is available, move the gels gently in the water once every minute.



9. *Wash Gels.* Perform a second wash as in step 3.



10. *Record Results.* Take a picture of your gel for analysis during the next class period.

Lesson 6 DNA Analysis by Gel Electrophoresis

Discussion Questions

(Adapted from Bio-Rad Biotechnology Explorer)

1. Explain the differences between an intron and an exon.

2. Explain how agarose electrophoresis separates DNA fragments. Why does a smaller DNA fragment move faster than a larger one?

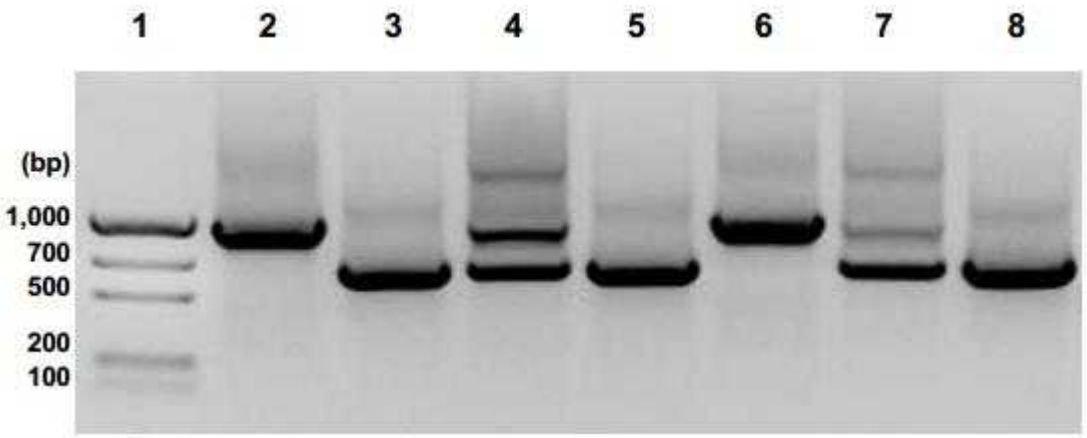
3. What kinds of controls are run in this experiment? Why are they important? Could others be used?

Lesson 7 Analysis and Interpretation of Your Results

Interpreting Your Gel

(Adapted from Dolan DNA Learning Center, Cold Spring Harbor Laboratory and Bio-Rad Biotechnology Explorer Image Source: www.bio-rad.org)

1. Determine your PV92 genotype. Practice with the gel below. Based on what you know about the *Alu* locus on chromosome 16, can you determine what genotype each lane displays? How do you know? Label each lane on the gel below and then use it as a key in determining the genotypes represented on your own gel photograph.



Other notes to keep in mind when analyzing your gel:

a. It is common to see a diffuse (fuzzy) band that runs ahead of the 121-bp marker. This is "primer dimer," an artifact of the PCR reaction that results from the primers overlapping one another and amplifying themselves. The presence of primer dimer, in the absence of other bands, confirms that the reaction contained all components necessary for amplification.

b. Additional faint bands at other positions occur when the primers bind to chromosomal loci other than the PV92 locus and give rise to "nonspecific" amplification products.

My sample shows band(s) at: _____

My *Alu* PV92 genotype is: _____

2. Why do the two possible PCR products differ in size by 300 base pairs?

3. Determine the observed genotype and allele frequencies for your class using the equations below. Determine what table best represents your data, draw it in the space below, and record your answers there.

$$\text{genotype frequency (\%)} = \frac{\text{number of students of X genotype}}{\text{total student samples}} \times 100$$

$$\text{allele frequency (\%)} = \frac{\text{number of X alleles}}{\text{total alleles in sample}} \times 100$$

Lesson 8 Molecular Techniques and Genetic Diseases/Disorders

Investigating Genetic Disorders

The medical field is now more capable of detecting genetic disorders through various testing techniques. Explore the links below to familiarize yourself with how PCR and agarose gel electrophoresis might assist medical experts in detecting different disorders. Also look through the following links to see if there are techniques in addition to electrophoresis that are of use in detecting genetic disorders.

https://www.inkling.com/read/robbins-cotran-pathologic-basis-of-disease-8th/chapter-5/molecular-diagnosis-of-genetic

http://advameddx.org/download/files/AdvaMedDx_DxInsights_FINAL(2).pdf

http://www.hhmi.org/biointeractive/human-genetics-new-guide-medicine

http://www.hhmi.org/biointeractive/medicine-genomic-era

http://www.hhmi.org/biointeractive/telltale-genes-charting-human-disease

Reflection

1. What are some examples of how gel electrophoresis can help detect a genetic disorder or disease?

2. What is another technique for detecting genetic disorders or diseases that was new to you? Please describe how the process works below.

3. In addition to determining genetic disorders, what might be other uses for running a gel electrophoresis?

Lesson 9 Pharmacogenomics

Pharmacogenomics Exploration

In the previous exercise, you ran an agarose gel electrophoresis in order to determine your genotype for the transposon *Alu* PV92. You could run an agarose gel electrophoresis to determine your genotype at any number of genetic locations for many different purposes. Review the following links to see how a person's genotype at specific single nucleotide polymorphisms (SNPs, which we explored at the beginning of this lesson) could influence the medical treatment he or she receives. Use the reflection questions below to help guide your exploration of these resources.

http://www.youtube.com/watch?v=-6TEfYZQZnw

http://learn.genetics.utah.edu/content/pharma/intro/

http://learn.genetics.utah.edu/content/pharma/snips/

http://learn.genetics.utah.edu/content/pharma/development/

Reflection

1. Give an example of how molecular techniques help personalize medical treatments. What are the benefits for tailoring medical treatments to a person's genotype for this particular disease? Are there any negative consequences?

2. In addition to designing medical treatments, what other reasons are there for knowing a person's genotype or genome?

3. What do you think about the challenges and issues in personalized medicine and Pharmacogenomics?

Lesson 10 Introduction to Genetic Based Diseases/Disorders

Genetic Disorders

The medical field has also been able to better diagnose and treat diseases because for many, we now better understand the genetic basis of the disease. A **genetic disorder** is a disease that is caused by an abnormality in an individual's DNA. Abnormalities can range from a small mutation in a single gene to the addition or subtraction of an entire chromosome or set of chromosomes. To learn more about different genetic disorders, browse through the following links. Again, use the reflection questions below to help guide your exploration of the resources.

http://www.biochemistry.org/Portals/0/Education/Docs/BASC10_full.pdf

http://learn.genetics.utah.edu/content/disorders/

http://www.chg.duke.edu/diseases/disorders.html

http://www.yourgenesyourhealth.org/

Reflection

1. Which genetic disorder or disease is of interest to you; which might you be interested in exploring further? Please describe it below.

2. Was there a disorder or disease described here that you previously did not know had a genetic basis? What else was new or surprising to you?

3. What are the different types of genetic problems that might lead to the formation of a genetic disorder or disease? Do you understand the molecular mechanism by which they occur?

Lesson 11 Genetic Disorder/Disease Presentations

Presentation Guidelines

In your group, use any of the previous resources you've explored and select a genetic disorder to learn more about and present to the rest of the class. You may select any disease listed on these sites. If there is one of interest to you that is not on this page, please check with your instructor first before researching the disease.

Your group will be presenting (10-15 minutes) to the class the following information:

How do people get the disease? Describe how the genetic mutation becomes incorporated into a person's genome, describe possible inheritance patterns.

What is the disease? Describe the genetic basis for the disease and what effects this genetic disorder has on the body and development.

What are the symptoms of the disease? Fully describe the direct effects of the genetic disorder, if not already covered in the areas above, and add any secondary effects and complications that may be experienced throughout a person's lifetime.

How do doctors diagnose the disease? Describe the characteristics doctors look for and the tests that may be run to determine diagnosis.

How is the disease treated? Describe the medications, therapies, and treatments for this disease, including (if provided) the dosages, timeline, costs, and possible side effects to be aware of related to the treatment.

Interesting facts about the disease? This category may include information related to how the disease was first discovered and characterized, how it got its name, and the incidence rate (i.e. "This disease occurs in about 1 out of every 100,000 people" or "10 children are diagnosed in the United States each year").

Your group must include at least **two scholarly sources** in addition to the website provided. Please be sure not to just plagiarize the recommended website. For assistance in identifying scholarly sources, please refer to: http://www.emich.edu/library/help/peerreview.php. Also, feel free to further explore http://learn.genetics.utah.edu to assist you in understanding any of the concepts you may encounter.

The **presentation** can take any form you like; PowerPoint, webpage, Prezi, song, video, or poster board, etc. But, please try to have some sort of visual. I prefer you <u>not</u> just get up and talk through each point.

Lesson 11 Genetic Disorder/Disease Presentations

Grading Rubric

(Reference: Rubric for Oral Presentations, New England Association of School and Colleges, Commission on Public Secondary Schools)

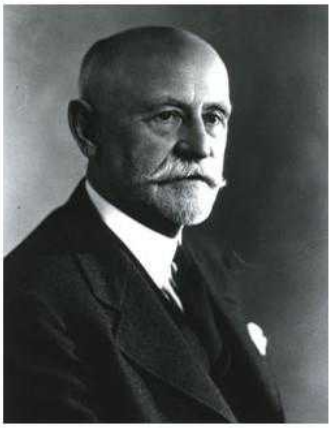
	Exceeds Standard	Meets Standard	Emerging	Attempt Made
Subject Knowledge	Demonstrates mastery of the topic in each of the following categories; what the disease is, how people get it, symptoms, diagnosis, treatment, and interesting facts.	Demonstrates accurate knowledge of the topic in each of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment	Demonstrates only some knowledge of the topic, and is missing one or two of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment	Demonstrates little knowledge of the topic, and is missing more than three of the following categories; what the disease is, how people get it, symptoms, diagnosis, and treatment
Organization and Coherence	Organizes information coherently, stays on the topic	Organizes most information, stays on the topic	Generally organizes information, occasionally strays from the topic	Poorly organizes information, often strays from the topic
Physical Presentation	Always speaks clearly/loudly, actively engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Usually speaks clearly/loudly, usually engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Speaks clearly/loudly, occasionally engages the audience by making and maintaining eye contact and using movement to focus attention/interest	Does not speak clearly/loudly, neglects to engage the audience, rarely makes and maintains eye contact or uses movement to focus attention/interest
Language Convention	Uses appropriate grammar and vocabulary	Mostly uses appropriate grammar and vocabulary	Makes some errors in grammar and vocabulary	Makes many mistakes in grammar and vocabulary
Visual Aids	Creatively uses a variety of effective visual aids and/or other methods of delivery	Uses visual aids moderately effectively and/or other methods of delivery	Moderately ineffective use of some visual aids and/or other methods of delivery	Does not use of visual aids and/or other methods of delivery
Scholarly Sources	More than two additional sources included and appropriately cited	Two additional scholarly sources included and appropriately cited	Either only one additional scholarly source included or not appropriately cited	Lacking two additional scholarly sources and not appropriately cited

Appendix F

Sample Student Final Presentation (begins next page)

Sickle Cell Anemia

Who Discovered Me?



James B. Herrick

Ernest Edward Irons

http://anemiacellsickle.weebly.com/history-of-disorder.html

In 1904, James B. Herrick clarified what sickle cell really is when his intern, Ernest Edward Irons, found sickle-shaped cells in the blood of Walter Clement Noel.

...Who Discovered Me?

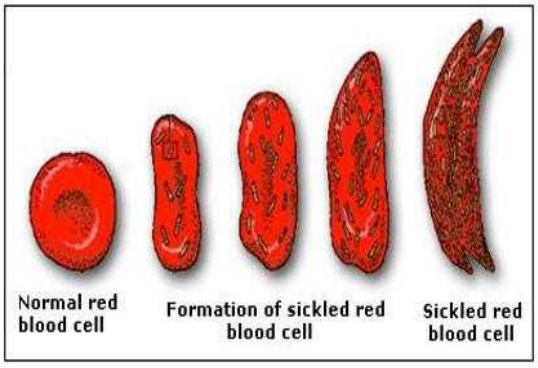
Walter Clement Noel was a 20 year old patient who was admitted to the Chicago Presbyterian Hospital. At the time, he was suffering from anemia. His hospitalization brought Ernest Irons to observe Noel's blood. In result, the discovery of sickle-shaped cells occurred.

In 1922, Verne Rheem Mason established this disease as "sickle-cell anemia." Its name was based on details Ernest Irons described to Mr. Mason.

http://www.cursumperficio.net/FicheAMMason.html

What Is Sickle Cell Anemia?

Sickle cell anemia is an inherited genetic disease that affects the formation of erythrocytes and various organs. Sickle Cell creates sickling in red blood cells which leads to pain and other serious symptoms.



http://www.tutorvista.com/content/biology/biology-iii/human-genetics/genetic-diseases.php

Mutations

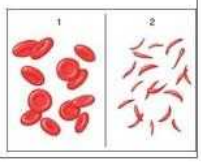
Our red blood cells are packed with Hemoglobin (the protein that carries oxygen). It has 4 proteins:

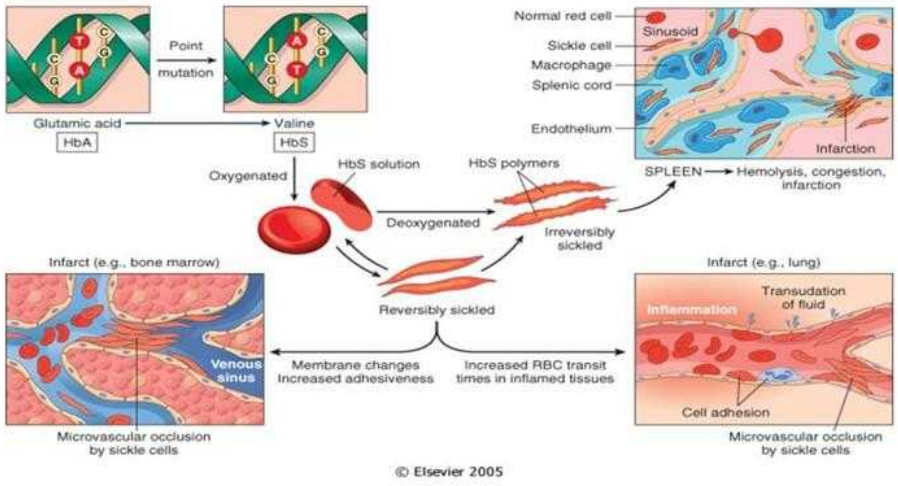
- 2 Beta chains
- 2 Alpha chains

One of the Beta chains, which is the 6th amino acid, gets mutated. That mutation creates the hemoglobin S (HbS).

In conditions of low oxygen, the mutated hemoglobin molecule (HbS) attracts to another mutated (HbS) molecule and they stick together.

- This mutation causes the hemoglobin to go from a round flexible shape to a rigid sickle shape.

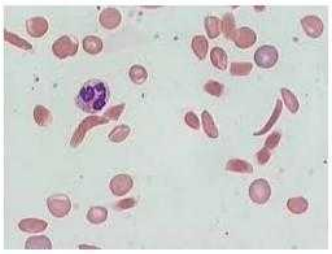




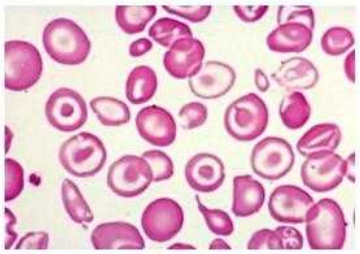
Beta-globin being replaced by hemoglobin S.

http://sgugenetics.pbworks.com/w/page/61172304/Pathophysiology%20of%20Sickle%20Cell%20Ane

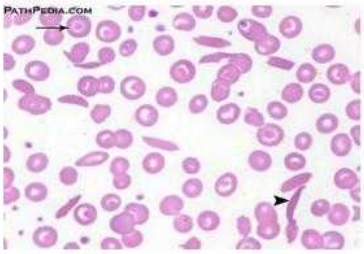
Types of Sickle Cell



Hemoglobin SS



Sickle Beta Thalassemia

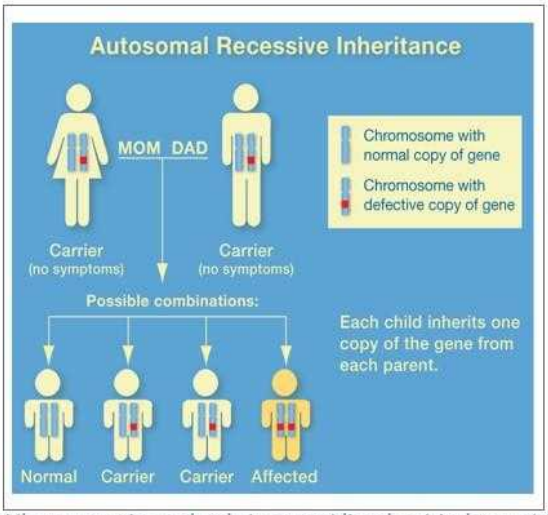


Hemoglobin SC

http://www.pathpedia.com/education/eatlas/histopathology/blood_cells/anemia-hemoglobin_sc_disease.aspx
http://www.med-ed.virginia.edu/courses/path/innes/rcd/hqbsyn.cfm
http://www.pathologystudent.com/?attachment_id=1247

There are three distinct types of sickle cell. This includes hemoglobin SS, hemoglobin SC, and Sickle Beta Thalassemia.

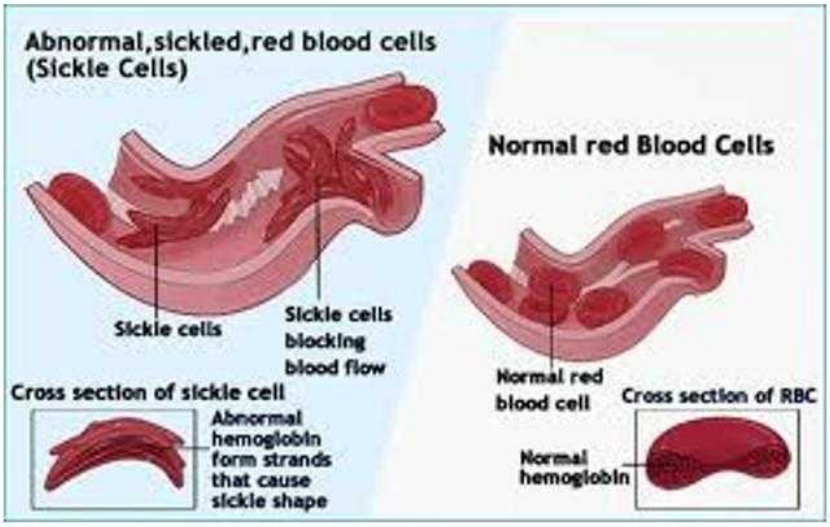
How do you get the disease?



If your mom and dad are sickle cell carriers, then there is a ...

- 25% chance that your blood will be normal
- 25% chance that you will have the sickle cell disease
- 50% chance that you'll be a carrier

http://learn.genetics.utah.edu/content/disorders/singlegene/sicklecell/



Do You See The Difference?

http://www.rudyard.org/sickle-cell-anemia-symptoms/

Testing & Screening

A blood test can check for the sickle cell anemia gene. In the U.S, this blood test is used on newborns as part of a routine screening.

- To test an adult, a blood sample is drawn from a vein in the arm.
- In young children and babies, the blood sample is collected from a finger.

The sample is then sent to a lab, where it will be screened for sickle cell anemia.

- If the screening test is *negative*, there is no sickle cell gene present.
- If the screening test is *positive*, further tests will be done to determine where one or two sickle cell genes are present.

Treatment

Bone marrow transplants are the only possible cure for sickle cell anemia, but finding a donor is difficult. The procedure has serious risks associated with it, including death.

Other Things That Relieve Pain Can Include..

- ❑ Hydroxyurea
- ❑ Blood Transfusions
- ❑ Antibiotics
- ❑ Heat packs
- ❑ Over-the-counter pain relievers
- ❑ Strong prescription pain medication

Children and Sickle Cell Disease: Annual Average Medical Expenses by Type of Insurance*

	Medicaid			Private Insurance		
	Children with SCD	Children without SCD	Costs due to SCD	Children with SCD	Children without SCD	Costs due to SCD
Inpatient admissions	$5,963	$359	$5,604	$7,820	$229	$7,591
Outpatient visits	$4,063	$349	$3,714	$6,371	$829	$5,582
Prescription drugs	$1,049	$317	$732	$531	$235	$296
Total expenses	$11,075	$1,706	$9,369	$14,722	$1,293	$13,469

*All expenses are average per child

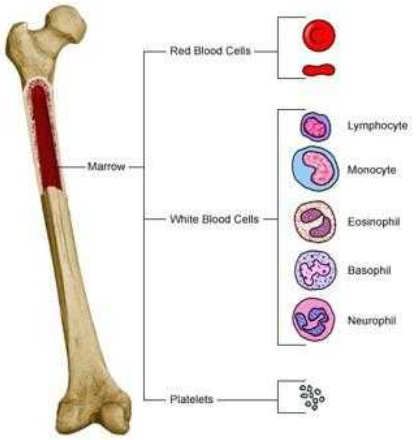
http://www.cdc.gov/Features/dsSickleCell_MedicalCosts/

The average total cost of care for patients with sickle cell anemia per month is $1,946.

Future Experimental Treatments

Gene Therapy

"Researchers are exploring whether inserting a normal gene into the bone marrow of people with sickle cell anemia will result in the production of normal hemoglobin." The idea came from the realization that sickle cell anemia is caused by a defective gene.



http://www.stardchnfoildrens.org/en/topic/default?id=bone-marrow-transplantation-in-children-90-P03062

....Gene Therapy

"Scientists are also exploring the possibility of turning off the defective gene while activating another gene responsible for the production of fetal hemoglobin — a type of hemoglobin found in newborns that prevents sickle cells from forming."

http://www.mayoclinic.org/diseases-conditions/sickle-cell-anemia/basics/treatment/con-20019348

Symptoms of Sickle Cell

Anemia causes...

★ Fatigue

Sickle cells, which die after 20 days, cause the body to have a shortage of red blood cells. Without enough red blood cells, your body can't get the oxygen it needs.

★ Episodes of Pain

Severe pain is caused when the sickle-shaped red blood cells block the flow of blood through blood vessels. This includes the following: chest, joints, bones, and abdomen. The crisis can be severe, leaving a person hospitalized.

★ Hand-Foot Syndrome

Usually, this is one of the first symptoms found in infants. This causes your feet and hands to swell up.

...Symptoms of Sickle Cell

★ Frequent Infections

Sickle cell can weaken your immune system, causing your body to be exposed to illnesses that you usually would not get. Due to this, doctors give special vaccinations and antibiotics to infants to help fight life-threatening infections.

★ Delayed Growth

Because of the shortage of healthy red blood cells, infants and children with sickle cell anemia have a delay in growing. This also includes puberty for teens.

★ Vision Problems

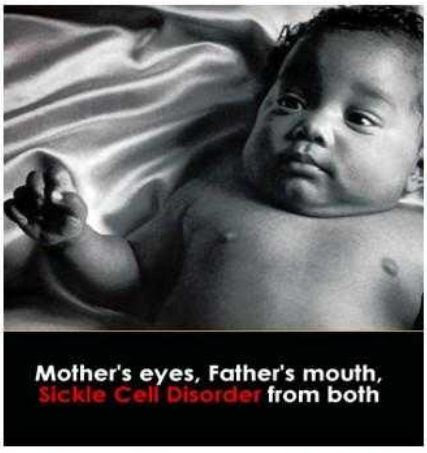
Blood cells that change shape can get trapped in blood vessels, blocking the blood flow. When this occurs in the small blood vessels in the inner lining (retina) of the eyes, it can cause vision problems.

Enjoy!

https://www.youtube.com/watch?v=R4-c3hUhhyc

Did You Know...

Over 72,000 Americans have sickle cell diseases.



http://www.pallenjones.com/ourvoice.html

1 in 150 to 1 in 2,000 newborns are diagnosed with sickle cell anemia.

Did You Know...

Because of James B. Herrick's contributions, "sickle cell anemia" was first named "Herrick's syndrome."

Even though sickle cell anemia is most commonly found in African Americans, it is possible for a person of any race or nationality to have the sickle cell trait.

Any Questions?

Sources

Stuart, MJ, and RL Nagel. "Sickle Cell Disease." *The Lancet*. 364(9442):1343-60., 9-15 Oct. 2004. Web. 14 Nov. 2014. <http://www.ncbi.nlm.nih.gov/pubmed/15474138?dopt=Abstract>.

Rees, D. C., Williams, T. N., & Gladwin, M. T. (2010, December 11). Sickle-cell disease. *The Lancet*, *376*(9757), 2018-2031. doi:10.1016/S0140-6736(10)61029-X

Slide 3 & 4) http://www.news-medical.net/health/Sickle-Cell-Disease-History.aspx

Slide 4 & 5) http://discovery.yukozimo.com/who-discovered-sickle-cell-anemia/

Slide 12) http://learn.genetics.utah.edu/content/disorders/singlegene/sicklecell/

Slide 8) http://ghr.nlm.nih.gov/condition/sickle-cell-disease

Slide 14 & 15) http://www.mayoclinic.org/diseases-conditions/sickle-cell-anemia/basics/symptoms/con-20019348
http://www.reuters.com/article/2009/07/03/us-sickle-cell-idUSTRE5623EL20090703

References

Achieve, Inc. (2010). *International science benchmarking report. Taking the lead in science education: Forging Next-Generation Science Standards*. Retrieved from http://www.achieve.org/files/InternationalScienceBenchmarkingReport.pdf.

Achieve, Inc. (2013). Next Generation Science Standards (Standards background: Research and reports). Achieve, Inc. Retrieved from http://www.nextgenscience.org/standards-background-research-and-reports.

ACT. (2012). The condition of college & career readiness. National. Retrieved from http://www.act.org/readiness/2012.

American Association for the Advancement of Science. (1993). *Benchmarks for science literacy*. New York, NY: Oxford University Press, Inc.

American Association for the Advancement of Science. (2007). *Atlas of science literacy, volume 2*. New York, NY: Oxford University Press, Inc.

American Institutes for Research. (2013). *Early college, early success: Early college high school initiative impact study*. Washington, DC: American Institutes for Research.

Anderson, R. D., & Helms, J. V. (2001). The ideal standards and the reality of schools: Needed research. *Journal of Research in Science Teaching*, 38(1), 3-16.

Anderson, R. D. (2002). Reforming science teaching: What research says about inquiry. *Journal of Science Teacher Education, 13(1),* 1-12.

Baram-Tsabari, A. & Yarden, A. (2005). Text genre as a factor in the formation of scientific literacy. *Journal of Research in Science Teaching*, 42(*4*), 403-428.

Berger, K. (1988). The developing person through the life span (2nd ed.). New York, NY: Worth Publishers Ltd.

Bell, R., Smetana, L., & Binns, I. (2005). Simplifying inquiry instruction. *The Science Teacher 72*(7): 30–34

Bigler, A. & Hanegan, N. (2011). Student content knowledge increases after participation in a hands-on biotechnology intervention. *Journal of Science Education and Technology*, 20(3), 246-257.

Bio-Rad Laboratories, Inc. (2016). *Biotechnology Explorer, Chromosome 16: PV92 Informatics Kit, 166-2100EDU*. Hercules, CA.

Bio-Rad Laboratories, Inc. (2016). *Biotechnology Explorer, STEM Electrophoresis Kit, 166-5080EDU*. Hercules, CA.

Biotechnology. (2015). In *Merriam-Webster.com*. Retrieved from http://www.merriam-webster.com/dictionary/biotechnology.

Blanchard, M., Southerland, S., Osborne, J., Sampson, V., Anetta, L., & Granger, E. (2010). Is inquiry possible in light of accountability? A quantitative comparison of the relative effectiveness of guided inquiry and traditional verification laboratory instruction. *Science Education*, *94*(4), 577-616.

Blumenfeld, P., Soloway, E., Marx, R., Krajcik, J., Guzdial, M. M., & Palincsar, A. (1991). Motivating project-based learning: Sustaining the doing, supporting the learning. *Educational Psychologist*, 26, 369-398.

Borgerding, L., Sadler, T., & Koroly, M. (2013). Teachers' concerns about biotechnology education. *Journal of Science Education and Technology*, *22*, 133-147.

Bransford, J. D., Brown, A. L., and Cocking, R. R., Ed. (2000). How People Learn: Brain, Mind, Experience, and School. Washington: National Academy Press.

Bredderman, T. (1982). What research says: Activity science—the evidence shows it matters. *Science and Children*, 20(1), 39-41.

Bredderman, T. (1983). Effects of activity-based elementary science on student outcomes: A quantitative synthesis. *Review of Educational Research*, *53*(4), 499-518.

Bronfenbrenner, U. (1994). Ecological models of human development. In *International Encyclopedia of Education, Vol 3, 2nd Ed.* Oxford: Elsevier.

Brown, D., Kemp, M., & Hall, J. (1998). Tough acts to follow: The challenges to science teachers presented by biotechnology progress. *International journal of Science Education*, 26, 717-733.

Bruner, J. (1960). The Process of Education. Cambridge, MA: Harvard University Press.

Bruner, J. (1961). The act of discovery. *Harvard Educational Review*, 31(*1*): 21-32.

Bruner, J. (1966). Toward a theory of instruction, Cambridge, Mass.: Belkapp Press.

Bruner, J. & Haste, H. (Eds.). (1987). Making sense: The child's construction of the world. New York, NY: Methuen.

Bruner, J. (1996). The Culture of Education. Cambridge, MA: Harvard University Press.

Bryce, T. & Gray, D. (2004). Tough acts to follow: The challenges to science teachers presented by biotechnological progress. *International Journal of Science Education*, 26, 717-733.

Buck, L.B., Bretz, S.L., & Towns, M.H. (2008). Characterizing the level of inquiry in the undergraduate laboratory. *Journal of College Science Teaching, 38*, 52-58.

Burns, M. (2007). *About Teaching Mathematics: A K-8 Resource*. Sausalito, CA: Math Solutions Publications.

Bybee, R. W. (1997). Achieving scientific literacy: from purposes to practices. Portsmouth, NH: Heinemann.

Bybee, R. & DeBoer, G. (1993). Goals for the science curriculum. In *Handbook of Research on Science Teaching and Learning*. Washington, DC: National Science Teachers Association.

Bybee, R. (2004). Scientific inquiry and science teaching. In L. Flick & N Lederman (Eds.). Scientific Inquiry and Nature of Science (pp. 1-14). Netherlands: Kluwer Academic Publishers.

Calhoun, E., Joyce, B., & Weil, M. (2004). Models of teaching (7th ed.). Boston, MA: Allyn and Bacon.

Carnegie Foundation. (2009). The opportunity equation: Transforming mathematics and science education for citizenship and the global economy. Carnegie Corporation of New York. Institute for Advanced Study. Commission on Mathematics and Science Education. Retrieved from http://opportunityequation.org/uploads/files/oe_report.pdf.

Castelló M., & Botella,L. (2006). Constructivism and educational psychology. In J. L. Kincheloe & R. A. Horn (Eds.), *The Praeger handbook of education and psychology* (Vol. 2, pp. 263-270). Westport, CT: Praeger. p. 263

Chaiklin, S. (2003) The zone of proximal development in Vygotsky's analysis of learning and instruction. In A. Kozulin, B. Gindis, V. Ageyev, & S. Miller (Eds.), Vygotsky's educational theory and practice in cultural context (pp. 39-64). Cambridge University Press.

Chen, R. & Raffan, J. (1999). Biotechnology: Students' knowledge and attitudes in the UK and Taiwan. *Journal of Biological Education*, 34, 17-23.

Chiappetta, E. (1997). Inquiry-based science. Strategies and techniques for encouraging inquiry in the classroom. *The Science Teacher*, 64(*7*), 22-26.

Cochran-Smith, M., & Lytle, S. (1990). Research on teaching and teacher research: The issues that divide. *Educational Researcher*, 19(2), 2-11.

Colburn, A. (2004). Inquiring scientists want to know. *Educational Leadership, 62(1),* 54-68.

Connell, J. (1994). *Reconstructing a pragmatic theory of knowledge: A transactional perspective*. Unpublished dissertation, University of Illinois, Urbana.

Crawford, B. (2007). Learning to teach science as inquiry in the rough and tumble of practice. *Journal of Research in Science Teaching*, 44(4), 613-642.

Dawson, V. (2007). An exploration of high school (12-17 year old) students' understandings of, and attitudes towards biotechnology processes. *Research in Science Education,* 37, 59-73.

Dawson, V. & Schibeci, R. (2003). Western Australian school students' understanding of biotechnology. *International Journal of Science Education,* 25, 57-69.

Dawson, V. & Soames, C. (2006). The effect of biotechnology education on Australian high school students' understanding and attitudes about biotechnology processes. *Research in Science and Technological Education,* 24, 183-198.

DeHaan, R. (2005). The impending revolution in undergraduate science education. *Journal of Science Education and Technology*, *14*(2), 253-270.

Dewey, J. (1910/1976). Science as subject-matter and as method. In J.A. Boydston (Ed.), *John Dewey: The middle works, 1899-1924* (Vol. 6, pp. 69-79). Carbondale, IL: Southern Illinois University Press.

Dewey, J. (1910). How we think. Lexington, MA: D.C. Heath.

Dewey, J. (1964). Science as subject-matter and as method. In (pp 182-192) Archambault RD (ed.) John Dewey on education: selected writings. Random House Inc, New York.

Dori, Y., Tal, R., & Tsaushu, M. (2003). Teaching biotechnology through case studies—can we improve higher order thinking skills of nonscience majors? *Science Education*, 87, 767–793.

Duckart, J. (1998). An evaluation of the Baltimore Community Lead Education and Reduction Corps (CLEAR Corps) program. *Evaluation Review*, *22*(3), 373-402.

Duffy, T. & Cunningham, D. (1996). Constructivism: Implications for the design and delivery of instruction, In D. H. Jonassen, (Ed.) Handbook of Research for Educational Communications and Technology, NY: Macmillan Library Reference USA.

Duit, R., & Treagust, D. (1998). Learning in science — From behaviourism towards social constructivism and beyond. In B. Fraser and K. Tobin (Eds) *International Handbook of Science Education* (pp. 3–26). Dordrecht: Kluwer Academic.

Edelson, D., Gordin, D., & Pea, R. (1999). Addressing the challenges of inquiry-based learning through technology and curriculum design. *Journal of the Learning Sciences*, 8(*3*), 391-450.

Edgar, D. (2012). *Learning theories and historical events affecting instructional design in education: Recitation literacy toward extraction literacy practices*. SAE open, 2, doi: 10.1177/2158244012462707

Ergül, R., Şimşekli, Y., Çaliş, S., Özdilek, Z., Gocmencelebi, Ş., & Şanli, M. (2011). The effects of inquiry-based science teaching on elementary school students' science process skills and science attitudes. *Bulgarian Journal of Science and Education Policy*, *5*(1), 48-68.

Ertepinar, H., & Geban, O. (1996). Effect of instruction supplied with the investigative-oriented laboratory approach on achievement in a science course. *Educational Research, 38(3),* 333-341.

Fay, M. & Bretz, S. (2008). Structuring the level of inquiry in your classroom. *The Science Teacher*, 38-42.

Fishman, B., Marx, R., Best, S., & Tal, R. (2003). Linking teacher and student learning to improve professional development in systemic reform. *Teaching and Teacher Education*, 19, 643–658.

Fleischman, H., Hopstock, P., Pelczar, M., and Shelley, B. (2010). Highlights from PISA 2009: Performance of U.S. 15-year-old student in reading, mathematics, and science literacy in and international context (NCES 2011-004). U.S. Department of Education, National Center for Education Statistics. Washington, DC: U.S. Government Printing Office.

Friedel, C., Irani, T., Rudd, R., Gallo, M., Eckhardt, E., & Ricketts, J. (2008). Overtly teaching critical thinking and inquiry-based learning: A comparison of two undergraduate biotechnology classes. *Journal of Agricultural Education*, *49*(1), 72-84.

Friesen, S. (2009). What did you do in school today? Teaching effectiveness: A framework and rubric. Toronto, ON: Canadian Education Association.

Friesen, S. (2013). *Inquiry-based learning: A review of the research literature*. Unpublished paper, University of Calgary.

Garcia, T., & Pintrich, P.R. (1992). Critical thinking and its relationship to motivation, learning strategies, and classroom experience. Paper presented at the Annual Meeting of the American Psychological Association, Washington, DC, August.

Gengarelly, L. & Abrams, E. (2009). Closing the gap: Inquiry in research and the secondary science classroom. *Journal of Science Education and Technology*, *18*: 74-84.

Gilbert, J. (2005). Catching the knowledge wave? The knowledge society and the future of education. Wellington, NZ: NZCER Press.

Gunter, B., Kinderler, J., & Beyleveld, D. (1998). Teenagers and biotechnology: A survey of understanding and opinion in Britain. *Studies in Science Education*, 32, 81–112.

Hanegan, N. & Bigler, A. (2009). Infusing authentic inquiry into biotechnology. *J Sci Edic Technol*, 18: 393-401. doi: 10.1007/s10956-009-9155-5

Haury, D. L. (1993). Teaching science through inquiry. ERIC clearinghouse for Science Mathematics and Environmental Education, ED359048, 1-6. Hofstein, A. and Lunetta, V. N. (2004). The laboratory in science education: Foundations for the twenty-first century. Sci. Ed., 88, 28-54.

Herman, J. & Knobloch, N. (2004). *Exploring the effects of constructivist teaching on students' attitudes and performance*. Proceeding of the 2nd Annual North Central Region AAAE Research Conference. Lafayette, IN: 21-35.

Herron, M. (1971). The nature of scientific inquiry. School Review, 79: 171-212.

Hofstein, A., & Lunetta, V. N. (2004). The laboratory in science education: Foundations for the twenty-first century. *Science Education, 88*(3), 28-53.

Hofweber, T. (2012). Logic and ontology. In: E. N. Zalta (Ed.), The Stanford encyclopedia of philosophy (Summer 2012 ed.). Retrieved from http://plato.stanford.edu/archives/sum2012/entries/logic-ontology/.

Hurd, P. D. (1991). Issues linking research to science teaching. *Science Education*, 75(6), 723-732.

Institute of Medicine, National Academy of Sciences, and National Academy of Engineering. (2007). *Rising above the gathering storm: Energizing and employing America for a brighter economic future*. Washington, D.C.: The National Academies Press.

Ketpichainarong, W., Panijpan, B., & Ruenwongsa P. (2010). Enhanced learning of biotechnology students by an inquiry-based cellulose laboratory. International Journal of Environmental & Science Education, 5(2): 169-187.

Kidman, G. (2010). What is an 'interesting curriculum' for biotechnology education? Students and teachers opposing views. *Research in Science Education*, 40, 353-373.

Klop, T., Severiens, S., Knippels, M., Hubertus, M., Van Mil, W., & Ten Dam, G. (2010). Effects of a science education module on attitudes towards modern biotechnology of secondary school students. *International Journal of Science Education*, 32(*9*), 1127-1150.

Knight, J. & Wood, W. (2005). Teaching more by lecturing less. *Cell Biology Education*, *4*(4), 298-310.

Krajcik, J.S., Blumenfeld, P., Marx, R.W., & Soloway, E. (2000). Instructional, curricular, and technological supports for inquiry in science classrooms. In J. Minstrell & E.H.V. Zee (Eds.), Inquiring into inquiry learning and teaching in science (pp. 283–315). Washington, DC: American Association for the Advancement of Science.

Kuklthau, C. C., Maniotes, L. K., & Caspari. A. K. (2007). Guided inquiry: Learning in the 21st century. Westport, CT & London: Libraries Unlimited.

Kuyper, H., van der Werf, M.P.C., & Lubbers, M.J. (2000). Motivation, meta-cognition and self-regulation as predictors of long term educational attainment. *Educational Research and Evaluation*, *6*(3), 181–201.

Kwon, H. & Change, M. (2009). Technology teachers' beliefs about biotechnology and its instruction in South Korea. *Journal of Technology Studies*, 35, 67-75.

Lee, O. (1989). Motivation to learning science in middle school classrooms. University Microfilms International. Unpublished doctoral dissertation, Michigan State University, East Lansing.

Lee, O., & Brophy, J. (1996). Motivational patterns observed in sixth-grade science classrooms. *Journal of Research in Science Teaching*, *33*(3), 585–610.

Leslie, G. & Schibeci, R. (2003). What do science teachers think biotechnology is? Does it matter? *Australian Science Teachers Journal*, 49(*3*), 16-21.

Leslie, G. & Schibeci, R. (2006). Teaching about designer babies & genetically modified foods: Encouraging the teaching of biotechnology in secondary schools. *The American Biology Teacher*, Online Publication, 98-103.

Lesmes Celorrio, M., Fernandez Gomez-Chacon, F., & Gonzalez-Soltero, R. (2013). An inquiry-based learning (IBL) approach to molecular biology for biotechnology undergraduate students. *Jornadas Internacionals de Innovacion Universitaria*.

Lewis, R. (2009). *Human genetics: Concepts and applications* (9th ed.). New York, NY: McGraw-Hill Companies, Inc.

Lock, R. & Miles, C. (1993). Biotechnology and genetic engineering: Students' knowledge and attitudes. *Journal of Biology Education*, 27, 267-272.

Macalalag, A., Brockway, D., McKay, M., & McGrath, El. (2010). *Partnership to improve student achievement in engineering and science education: Lessons learned in year one*. Hoboken, NJ: American Society for Engineering Education Mid-Atlantic.

Mansius, A. & Hanegan, N. (2008). Factors concerning integration of biotechnology into secondary biology. Paper presented at American Educational Research Association, New York.

Manus, A. L. (1996). Procedural versus constructivist education: A lesson from history. *The Educational Forum*, 60(4): 312-16.

Marx, R.W. (2003, April). Partnerships for urban systemic reform: The effects of inquiry curriculum developed by the Center for Learning Technologies in Urban Schools. Presented at the Annual Meeting of the American Educational Research Association, Chicago, IL.

Marx, R., Blummenfeld, P., Krajcik, J., Fisherman, B., Soloway, E., Geier, R., & Tal, R. (2004). Inquiry-based science in the middle grades: Assessment of learning in urban systemic reform. *Journal of Research in Science Teaching*, 41(10), 1063-1080.

McComas, W. (n.d.). Enhancing the education of scientifically gifted student with inquiry instruction. Presented at the USC Summer Institute for Teachers of the Gifted. University of Southern California Rossier School of Education. Los Angeles, CA.

Michael, M., Grinyer, A, & Turner, J. (1997). Teaching biotechnology: Identity in the context of ignorance and knowledgeability. *Public Understanding of Science*, 6, 1-17.

Michigan Department of Education. (2009). *Career and technical education standards*. Retrieved from http://ctenavigator.org/programs/program/38

Minner, D., Levy, A., & Century, J. (2010). Inquiry-based science instruction - What is it and does it matter? Results from a research synthesis year 1984 to 2002. *Journal of Research in Science Teaching*, *47*(4), 474-496.

Napier, J.D., & Riley, J.P. (1985). Relationship between affective determinants and achievement in science for seventeen-year-olds. *Journal of Research in Science Teaching*, *22*(4), 365–383.

National Academy of Sciences. (1996). *National Science Education Standards*. Washington, DC: National Academy of Sciences – National Research Council.

National Association of State Directors of Career Technical Education Consortium. (2006). Sample plans of study, Career clusters. Retrieved from http://www.careertech.org/career-clusters/.

National Center for Education Statistics. (2011). The Nation's Report Card: Science 2009 (NCES 2011-451). Institute of Education Sciences, U.S. Department of Education, Washington, D.C.

National Center for Education Statistics. (2012). The Nation's Report Card: Science 2011 (NCES 2012-465). Institute of Education Sciences, U.S. Department of Education, Washington, D.C.

National Consortium for Health Science Education - NCHSE. (2015). National Health Science Standards. Retrieved from http://www.healthscienceconsortium.org/wp-content/uploads/2015/07/NATIONAL-HEALTH-SCIENCE-STANDARDS-May-2015f1-PE2.pdf

National Research Council. (1996). *National Science Education Standards*. Washington, D.C.: National Academy Press.

National Research Council. (1999). *Designing mathematics or science curriculum programs: A guide for using mathematics and science education standards*. Washington, D.C.: The National Academies Press.

National Research Council. (1999). *Selecting instructional materials: A guide for K-12 science*. Washington, D.C.: The National Academies Press.

National Research Council. (2000). *Inquiry and the National Science Education Standards: A Guide for Teaching and Learning*. Washington, D.C.: National Academies Press.

National Research Council. (2005). *America's lab report: Investigations in high school science*. Washington, D.C.: The National Academies Press.

National Research Council. (2007). *Taking science to school: Learning and teaching science in grades K-8*. Washington, D.C.: The National Academies Press.

National Research Council. (2012). *A framework for K-12 science education: Practices, crosscutting concepts, and core ideas*. Washington, DC: The National Academies Press.

National Science Teachers Association. (1992). *Volume I: The content core, a guide for curriculum designers*. Washington, DC: Author.

National Science Teachers Association. (2004). *NSTA position statement: Scientific Inquiry*. Official Positions. Retrieved from http://www.nsta.org/pdfs/PositionStatement_ScientificInquiry.pdf.

NGSS Lead States. (2013). *Next generation science standards: For States, by States*. Washington, D.C.: The National Academies Press.

New England Association of Schools and Colleges, Inc. (2016). *2011 standards for accreditation: Revised*. Burlington, MA.

Olsher, G. (1999). Biotechnologies as a context for enhancing junior high school students' ability to ask meaningful questions about abstract biological processes. *International Journal of Science Education,* 21(*2*), 135-153.

Olsher, G. & Dreyfus, A. (1999). Biotechnologies as a context for enhancing junior high-school students' ability to ask meaningful questions about abstract biological processes. *International Journal of Science Education,* 21, 137–153.

Ormrod, J. (2012). Essentials of educational psychology: Big ideas to guide effective teaching. Boston, MA: Pearson Education Inc.

Oxford, R. (1997). Constructivism: Shape-shifting, substance, and teacher education. *Peabody Journal of Education*, 72(1): 35-66.

Ozel, A., Terzi, I., & Ozel, E. (2009). Awareness of biological application: A study among university geography students. *Education*, 129, 714-723.

Pacific Policy Research Center. (2010). *21st Century Skills for Students and Teachers*. Honolulu: Kamehameha Schools, Research & Evaluation Division.

Partnership for 21st Century Learning. (2007). *Framework for 21st century learning*. Washington, DC.

Penuel, W., Harris, C., & Haydel DeBarger, A. (2015). Implementing the Next Generation Science Standards. Phi Delta Kappan, 96(6), 45-49.

Piaget, J. (1964). Development and learning. In. R.E. Ripple & V.N. Rockcastle (Eds.), Piaget Rediscovered: A Report on the Conference of Cognitive Studies and Curriculum Development (pp. 7-20). Ithaca, NY: Cornell University.

Pintrich, P.R., Marx, R.W., & Boyle, R.A. (1993). Beyond cold conceptual change: The role of motivational beliefs and classroom contextual factors in the process of conceptual change. *Review of Educational Research, 63*(2), 167–199.

Plein, L. C. (1991). Popularizing biotechnology: The influence of issue definition. *Science, Technology, & Human Values*, 16(4), 474-490.

Prokop, P., Leskova, A., Kubiatko, M., & Diran, C. (2007). Slovakian students' knowledge of and attitudes toward biotechnology. *International Journal of Science Education*, 29, 895-907.

Reece, J., Taylor, M., Simon, E., & Dickey, J. (2010). *Campbell biology* (9th ed.). San Francisco, CA: Benjamin Cummings

Reicks, M., Stoebner, T., Hassel, C., & Carr, T. (1996). Evaluation of a decision case approach to food biotechnology education at the secondary level. *Journal of Nutrition Education and Behavior*, 28, 33-38.

Roth, W-M., McGinn, M. K., & Bowen, G. M. (1998). How prepared are preservice teachers to teach scientific inquiry? Levels of performance in scientific representation practices. *Journal of Science Teacher Education, 9*, 25-48.

Schwab, J. (1960). What do scientists do? *Behavioral Science*, 5(*1*), 1-27.

Schwab, J. (1966). The teaching of science. Cambridge, MA: Harvard University Press.

Schwerdt, G. & Wuppermann, A. (2010). *Is traditional teaching really all that bad? A within-student between-subject approach* (Working Paper No. PEPG 10-15). Retrieved from Program on Educational Policy and Governance Harvard Kennedy School website: http://www.hks.harvard.edu/pepg/

Shuell, T. (1993). Toward an integrated theory of teaching and learning. *Education Psychologist*, 28(*4*): 291-311.

Shymansky, J. A., Kyle, W. C. Jr., & Alport, J. M. (1983). The effects of new science curricula on student performance. *Journal of Research in Science Teaching, 20(5),* 387-404.

Simon, M. & Schifter, D. (1991). Toward a constructivist perspective: An intervention study of mathematics teacher development. *Educational Studies in Mathematics*, *22*(4), 309-331.

Singer, J., Marx, R.W., Krajcik, J.S., & Clay-Chambers, J. (2000a). Constructing extended inquiry projects: Curriculum materials for science education reform. *Educational Psychologist*, 35, 165–178.

Socratic Method. (2013). In *Merriam-Webster.com*. Retrieved from http://www.merriam-webster.com/dictionary/socratic%20method.

Soloway, E., Guzdial, M., & Hay, K. (1994). Learner-centered design: The challenge for HCI in the 21st century. *Interactions*, 1, 36–48.

Spronken-Smith, R., Bullard, J., Ray, W., Roberts, C. and Keiffer, A. (2007). Where might sand dunes be on Mars? Engaging students through inquiry-based learning in geography. *Journal of Geography in Higher Education* (in press).

Spronken-Smith, C., Bullard, J., Ray, W., Roberts, C., & Keiffer, A. (2008). Where might sand dunes be on Mars? Engaging students through inquiry-based learning in geography. *Journal of Geography in Higher Education*, 32(1), 71-86.

Steele, F. & Aubusson, P. (2004). The challenges in teaching biotechnology. *Research in Science Education*, *34*, 365-387.

Strike, K.A., & Posner, P.J. (1983). On rationality and learning: A reply to West and Pines. *Science Education, 67*, 41–43.

Strike, K.A., & Posner, P.J. (1992). A revisionist theory of conceptual change. In R.A. Duschl and R.J. Hamilton (Eds) *Philosophy of Science, Cognitive Psychology, and Educational Theory and Practise* (pp. 147–176). Albany, NY: State University of New York Press.

Sullivan, G. & Artino, A. (2013). Analyzing and interpreting data from Likert-type scales. *Journal of Graduate Medical Education*, *5*(4), 541-542.

The College Board. (1999). Released exam: 1999 AP Biology. n.p. n.d. Web. 29 Oct. 2014.

Tuan, H., Chin, C., & Shieh, S. (2005). The development of a questionnaire to measure students' motivation towards science learning. *International Journal of Science Education, 27*(6), 639-654.

Turkmen, L. & Darcin, E. (2007). A comparative study of Turkish elementary and science education major students' knowledge level at the popular biotechnological issues. *International journal of Environmental Science Education*, 2, 125-131.

United States. National Commission on Excellence in Education. (1983). *A nation at risk: The imperative for education reform: A report to the Nation and the Secretary of Education, United States Department of Education*. Washington, D.C.: The Commission: [Supt. of Docs., U.S. G.P.O. distributor]

Venville, G. & Treagust, D. (2002). Teaching about the gene in the genetic information age. *Australian Science Teachers' Journal*, 48(*2*), 20-24.

von Glasersfeld, E. (1989). Constructivism in education. In: T.Husen and T. Neville Postlethwaite (eds) *The international encyclopedia of education*. Research and Studies, pp. 162-163. Supplementary Volume 1. Oxford: Pergamon Press.

Von Secker, C, & Lissitz, R. W. (1999). Estimating the impact of instructional practices on student achievement in science. *Journal Research in Science Teaching*, 56(10), 1110-1126.

Vygotsky, L. S. (1978a). Interaction between Learning and Development. In M. Cole, V. John-Steiner, S. Scribner, & E. Souberman (Eds.), *Mind in society: the development of higher psychological processes* (pp. 79-91). Harvard University Press.

Vygotsky, L.S. (1978b). Mind in Society. Cambridge, MA: Harvard University Press.

West, L.H.T., & Pines, A.L. (1983). How 'rational' is rationality? *Science Education, 67*, 37–39.

White, S. & Dillow, S. (2005). *Key concepts and features of the 2003 National Assessment of Adult Literacy* (NCES 2006-471). U.S. Department of Education, National Center for Education Statistics. Washington, DC: U.S. Government Printing Office.

Wilke, R. & Straits, W. (2005). Practical advice for teaching inquiry-based science process skills in the biological sciences. *The American Biology Teacher*, 67(9), 534-540.

Windschitl, M. (2002). Inquiry projects in science teacher education: What can investigative experiences reveal about teacher thinking and eventual classroom practice? *Science Education, 81*(1), 112-143.

Windschitl, M. (2004). Folk theories of 'inquiry": How preservice teachers reproduce the discourse and practices of an atheoretical scientific method. *Journal of Research in Science Teaching*, *41*(5), 481-512.

Witt, C. & Ulmer, J. (2010). The impact of inquiry-based learning on the academic achievement of middle school students. Western AAAE Research Conference Proceedings, 269-282.

Wolters, C.A. (1999). The relation between high school students' motivational regulation and their use of learning strategies, effort, and classroom performance. *Learning and Individual Differences*, *11*(3), 281–300.

Zeller, M. (1994). Biotechnology in the high school biology curriculum: The future is here! *American Biology Teachers*, 56, 460-464.

Zion, M. & Mendelovici, R. (2012). Moving from structured to open inquiry: Challenges and limits. *Science Education International*, *23*(4), 383-399.