Beyond Mendel

How current plant genetics is advancing with the use of quantitative genetics.

Quantitative genetics

BEYOND MENDEL

Author: Wendy Vidor

Curriculum Team: Houda Darwiche, Drew Joseph, Mary Jo Koroly

Lesson Two Adapted from REDNEXTRACT DNA/PCR Protocol by Sigma Aldrich

Thank you to the following who offered excellent review and suggestions:

Dr. Mathias Kirst

Dr. Kelly Balmant

Dr. Annette Farhenkrog

University of Florida Forest Genomic Research Team

Please direct inquiries to Julie Bokor at Julie@cpet.ufl.edu or 352.392.2310.

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Center for Precollegiate Education and Training

PO Box 112010 • Yon Hall, Room 331

Gainesville, FL 32611

Phone 352.392-2310• Fax 352.392-2311

This curriculum was developed in the laboratory of:

Matias Kirst, PhD
Professor
School of Natural Resources and Conservation
College of Agriculture and Life Science

Matias Kirst joined the School of Forest Resources and Conservation in 2005 as an Assistant Professor in Quantitative Genetics. In addition to his affiliation to the SFRC, he is also a member of the Plant Molecular and Cellular Biology Program (PMCB) and the University of Florida Genetics Institute (UFGI). His group in Quantitative Genomics Research is part of the Forest Genomics Laboratory. Research is focused in three areas: (1) Fundamental Genomic Research in the genetic regulation of gene expression and gene expression networks; (2) Applied Genomic Research for the discovery of genes, metabolic and regulatory networks that control variation in wood quality, growth and other important traits for the forestry and agronomic industry; and (3) Technology and genomic tool development

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AUTHOR'S NOTE

I am completing my 3-week fellowship in the Forest Genomic Research Lab with Dr. Mathias Kirst. I was first approached to the possibility of learning about genetics research and the applications towards climate change and bioenergy with trees. It was introduced at the Bench to Bedside Workshop in 2015.

I currently am a doctoral candidate in Environmental Horticulture emphasizing Plant Biotechnology and thought that this would be a great learning experience to help me in my dissertation research and most importantly to build some current curriculum with newer technology that I could introduce to my Agriculture Biotechnology students.

I met with Dr Kirst later in the summer and we talked about the possibility to work on this curriculum portion as part of a grant. He invited me to work in the lab and the CPET staff accepted my application to attend the research experience.

I am very excited to have this opportunity to learn about genomic qualitative research because of all the practical applications it can hold for agriculture and horticulture applications. My learning experience in the laboratory and the graduate students research and knowledge that they have shared is invaluable as an educator. I believe this is one of the best professional development experiences I attended.

Genetics is an exciting field of study and offers many opportunities for students to pursue in the areas of medicine, agriculture, biotechnology and horticulture. Using qualitative genetics, we can look closely at associations of genes that control phenotypic traits and use that to our advantage in agriculture to control disease, breeding programs, bio energy and food sources. I believe this will provide more opportunities for students to pursue horticulture careers and emphasize their necessity to fund applications with new technology. There are many new career pathways that will be open for students with an agriculture/biotechnology background and apply in these new fields.

INTRODUCTION

In the field of biotechnology many new techniques and advances have occurred due to the sequencing of the human genome. Current technology is outpacing existing technology especially in genetics. Qualitative genetics is an area that studies non-medallion traits to focus on phenotype changes. Current research is focused on linking phenotypes to applied applications in agriculture, medicine and other industry.

The lessons in this unit provide information on using biotechnology applications in fundamental genomic research. Students will be investigating current technologies in Next Generation Sequencing such as real-time PCR using quantitative models (complex non -mendellian traits), and analyzing gene sequences of *Pisium sativum genetic seed stock.* The investigation will help students look into these new technologies being used in genomic research today.

These quantitative traits of study are looking closely at phenotype classes that are not clearly distinguished and variation in in degree rather than kind. The effect of many genes contribute to the phenotypic variation with small additive effects. Variation is caused by both environment playing a -major role in plants and genetics. Phenotype = Genetics + Environment.

The use of genetic markers which are places in the chromosome where individuals are different. The types of markers used in qualitative research are single nucleotide polymorphism or SNP's with is a mutation in a single base variation. This usually the variation between individuals (alleles) involve just a single base A, G. SNP's have the most common form in genetic variation. We will investigate these methods which are QTL analysis and Illuminum Infinnium or single base extension. The technology is leading to whole genome sequencing in the future.

The analysis of these phenotypes are measured with linkage disequilibrium. The allele frequency at a specific loci position. As future generations occur, shorter regions on alleles appear due to recombination. These factors during meiotic reproduction include independent assortment and crossing over. If the alleles at 2 loci are not independent of one another they may have some correlation. That is where researchers can use SNP's as genetic markers for studies on what the gene may be controlling. They are looking for genes that control traits.

There are new analysis techniques using these genetic markers to tell which parent contributes what piece of the chromosome. These associations of a trait on a specific locus may be controlling the trait. Quantitative Trait Loci Analysis and Association Genetic Analysis are two current methods that are looking at these quantitative traits.

The goal for agriculture and plant biotechnology is to identify certain breeding crosses to improve or make new products, find diseases and other applications to improve our lives.

TIPS ABOUT THIS CURRICULUM

This curriculum is designed for a biotechnology course or an AP Biology course. The students will need to grow the pea plant genotypes at least 4 weeks before the unit. The sequence must be followed as written. The teacher will need to send the PCR product to the lab for sequencing. It is suggested the students begin the extension activity or research while waiting for the sequence to return from the lab.

Please allow for 3 to 4 class periods to complete the DNA extraction and PCR. Give students the opportunity to review the video clip provided with links before the lab activities.

LESSON PLAN FORMAT

Lesson Plan Format: All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

KEY QUESTION(S): Identifies key questions the lesson will explore.

OVERALL TIME ESTIMATE: Indicates total amount of time needed for the lesson, including advanced preparation.

LEARNING STYLES: Visual, auditory, and/or kinesthetic.

VOCABULARY: Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.

LESSON SUMMARY: Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.

STUDENT LEARNING OBJECTIVES: Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

STANDARDS: Specific state benchmarks addressed in the lesson. Also collected in one list.

MATERIALS: Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.

BACKGROUND INFORMATION: Provides accurate, up-to-date information from reliable sources about the lesson topic.

ADVANCE PREPARATION: This section explains what needs to be done to get ready for the lesson.

PROCEDURE WITH TIME ESTIMATES: The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

ASSESSMENT SUGGESTIONS: Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/posttest) that can be given. Teachers should feel free to create additional formative and summative assessment pieces. Laboratories will include a rubric and extension questions.

EXTENSIONS: (ACTIVITIES/LITERATURE) There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.

RESOURCES/REFERENCES: This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available. All references and resources are also collected in one list.

STUDENT PAGES: Worksheets and handouts to be copied and distributed to the students.

TEACHER MASTERS: Versions of the student pages with answers or the activity materials for preparation.

Collaborative Learning: The lessons in this curriculum have been developed to include many collaborative learning opportunities. Rather than presenting information in lecture format and teacher driven, the activities involve the students in a more engaged manner. For classrooms not accustomed to using collaborative learning strategies, have patience. It can be difficult to communicate instructions, particularly for students who are visual learners. For these students, use of visual clues such as flowcharts and graphics can help them understand how they are to move to different groups.

Groups: Most of the lessons are carried out in groups. While it isn't necessary for students to remain in the same groups the entire unit, if they work well together, it may foster students to think deeper as they are comfortable with their teammates and willing to ask questions of each other.

Inquiry-based: The lessons in the curriculum invite students to be engaged and ask questions. They work through background information in a guided fashion, but are challenged to think beyond what they have read or done. The teacher serves as the facilitator in these activities, not the deliverer of information.

Technology: Lessons have been written to be mindful of varying availability of technology in schools and homes. Some of the lessons would be very well suited to online environments and if your students are able, you might wish to engage in some of the technology modifications. Grow lights or a greenhouse to grow out the plants will be necessary if students will complete the laboratory activities.

Content:

Implementation Notes: Students will need to grow out the pea plants 4 weeks ahead of time in the greenhouse or grow chamber. The pea genotypes will be included with a PCR kit that will be available from the Forest Genomics Lab.

Extensions: Students should be encouraged to do an Agriscience Fair Project and Research Project looking at the effects of the identified mutations on the phenotypes and submit their projects during the state FFA science fair.

Science and Subject: Plant Biotechnology ability level: 11-12, with previous biotechnology courses level 2 and 3.

Science concepts:

Genetic markers, gene sequencing, single nucleotide polymorphisms, qualitative trait loci analysis, polymerase chain reaction for DNA, DNA purification, SNP analysis and association analysis.

Lesson 1:

Why do giraffes have long necks?

What are SNP's?

Students will build background knowledge on investigating quantitative genetics in a reading activity. They will be able to read a real application of phenotypic mutations in genes of a giraffe. They will complete the reading according to reading strategy using individual Lexile levels. They will answer questions from the reading using software from www.Newsela.com. This reading will be the hook for students to investigate current technologies in the use of genetic markers and quantitative genetics to study how genes control certain phenotypic traits. This activity sets the stage for students to perform current technologies of looking at phenotypes using both Mendellian and quantitative traits. They will then use an activity called "What is a SNP" as a virtual lab activity.   This lesson will challenge students to look at genetics from a current research point of view utilizing current biotechnological procedures. In this lesson, students will understand the basis of the environmental and genetic factors on phenotypic polymorphisms or variation, and know how we use SNP's as genetic markers for investigation of these changes to an organism.

Lesson 2: DNA Extraction of Mendel's Peas

Part 2: PCR of Mendel Pea Strains and PCR Purification and sequence analysis

Working in groups, students will review the video clip on Genomic DNA Plant Extraction: They will take notes in the lab notebooks documented the steps needed to extract the DNA from the *Pisium sativium* plants.

Lesson 3: Bioinformatics: Analyzing the Aradopsis Sequences

Working in groups, students will do a practice worksheet on genetic recombination. They will then be introduced to the bioinformatics program CLUSTALW. They will be given their sequenced material from the UF Forestry Genomics Lab and they will analyze their sequence to see what variations they have from their PCR Samples. Students will then match their samples to the genotype of the plants they grew in the greenhouse. They will see what loci the gene is associated with on the chromosome. They can then recognize the SNP's or genetic markers of this variation.

Lesson 4: Extension Activity: Agriscience Research Project

An extension is to have the students complete an agriscience fair project or research paper that can contribute to agriculture, such as biofuel production, or breeding to present at the FFA agriscience state competition.

LESSON SEQUENCING GUIDE

Since the classroom teacher knows his or her students best, the teacher should decide the sequencing of lessons. Below is a suggested pacing guide that can be used when planning to use this curriculum. 50 Minute Class Periods

	Day 1	Day 2	Day 3	Day 4	Day 5
Week 1	Lesson 1 Flipping Exercise (Homework) Standing Tall (30 minutes) What is a SNP? (20 minutes)	Lesson 1 Part 2 Flipping Exercise (Homework)	[A]Lesson 2 Part 1 DNA Extraction (50 minutes)	Lesson 2 PCR: Part 2 Prepare DNA for Sequencing (50 minutes)	Lesson 4 Bioinformatics Analyze the Sequence (45 minutes)
Week 2	Extension Lesson 5 Agriscience Research Project (To be added later)				

[A] Lesson 1– SNP's can be adapted for use outside of the classroom period with simulation for homework and/or discussions taking place through web-based platforms

[B] Lesson 3 – In preparation for the BLAST Sequencing Activity, students will be beginning their extension activity as it will take a week to get the sequences back from the lab.

[C] Lesson 5 – The extension activity can be utilized during the period the sequences are analyzed.

Alleles: Place on a chromosome that contains genes from a parent.

Association Genetics: Is the process of looking at association of small pieces of genetic information on a locus after several generations of reproduction and using the information to predict a quantitative trait for further research.

Cell Lysis: The breaking down of the membrane of a cell using viral, enzymic or osmotic mechanisms that compromise the cell wall integrity.

Crossing over: an interchange of genes or segments between homologous chromosome

Deletion: removal of one or more nucleotides from a DNA sequence, which may alter the reading frame

DNA: Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms.

DNA Isolation: a process of purification of DNA from a sample using a combination of physical and chemical methods.

DNA Sequencing: Is any method used to determine the precise order of nucleotides within a DNA molecule.

Elution Buffer: a method to remove the wash buffer using water or Nuclease free water in which is added to the column and filtered through a membrane. The buffer removes the nucleic acid from the membrane and is collected at the bottom of the column.

Enzyme: Enzymes are proteins that catalyze (i.e., increase the rates of) chemical reactions. Almost all chemical reactions in a biological cell need enzyme in order to occur at rates sufficient for life.

Genomic DNA: Deoxyribonucleic acid is a nucleic acid containing genetic instructions used in the development and functioning of all know living organisms.

Genotype: The genotype is the genetic makeup of a cell, an organism, or an individual (i.e. the specific allele makeup of the individual). The genotype of an organism is the inherited instructions it carries within its genetic code.

Genetic Markers: A gene or short sequence of DNA to identify a chromosome or locate other genes or specific positons on a chromosome.

Haplotype: Describes the alleles at multiple loci received from each parent.

Homologous Chromosomes - Each individual inherits one copy (haploid) of each chromosome from its parents to for a diploid organism.

Insertion: addition of one or more nucleotides in a DNA sequence, which may alter the reading frame

Linkage Disequilibrium: The non-random association of genes at loci. They are at disequilibrium when the frequency of association of their different alleles is higher or lower than what would be expected if the loci were independent and associated randomly.

Linkage map: a representation of the relative positions of genes in a chromosome
Locus (Loci): A specific location or position of a gene's DNA sequence, on a chromosome.

Lysis Buffer: is a buffer solution used for breaking open cells for used in molecular biology to analyze the compounds of cells using salts to regulate acidity and osmolarity of the lysate.
Washing Buffer

Phenotype: An organism's observable characteristics or traits. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two.

Polymerase Chain Reaction: A technique used to amplify a copy or copies of a DNA generating thousands or millions of copies of that sequence.

Quantitative Genetics: A branch of genetics that vary continuously varying characters and rather than focusing on changes in frequency of specific allele genotypes, it quantifies changes in the frequencies of specific alleles of traits that cannot be placed into discrete phenotypic classes.

Recombination: the processes of crossing-over and independent assortment of new combinations of genes in offspring that did not occur in the parents

Segregation of Alleles: Each progeny receives one allele from each parent.

Single Nucleotide Polymorphisms: A variation in a single nucleotide that occurs at a specific position in the genome where each variation is present

Benchmark:	Lesson						
	1	2	3	4	5	6	7
17.10 Analyze factors that influence gene expression. SC.912. L. 16.5, 16.6							
17.11 Describe the process of genetic marker assisted selection. LAFS. SL 2.4; SC912.L.16.7							
23.05 Conduct a polymerase chain reaction using an agricultural product. SC.912.L.16.12							
19.14 Explain biomass and sources of biomass. SC.912.L. 17.11							
19.15 Assess the characteristics of biomass that make it useful for biofuels production. SC.912.L.18.7, 8, 9;							
24.08 Use web-based resources to find information on the genetic sequence of a protein using bioinformatics. LAFS.1112.RI.3.7; SC.912.L.18.4							
34.03 Describe how genetic processes and structures control inheritance in plants. LAFS.1112.SL.2.4, LAFS.1112.L.3.6; SC.912.L.16.1, 2, 4;							
34.05 Differentiate phenotypic versus genotypic expression in plant crosses. SC.912.L.16.1, 2, 4;							
38.02 Analyze the scope and impact of plant biotechnology in today's global society. SC.912.L.16.10; SC.912.N.4.2							
Science and Language Arts Standards Covered:							
SC.912.L.16.1 Use Mendel's laws of segregation and independent assortment to analyze patterns of inheritance.							
SC.912.L.16.2 Discuss observed inheritance patterns caused by various modes of inheritance, including dominant, recessive, codominant, sex-linked, polygenic, and multiple							

Benchmark:	Lesson						
	1	2	3	4	5	6	7
alleles.							
SC.912.L.16.3 Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.							
SC.912.L.16.4 Explain how mutations in the DNA sequence may or may not result in phenotypic change. Explain how mutations in gametes may result in phenotypic changes in offspring.							
SC.912.L.16.7 Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.							
SC.912.L.16.9 Explain how and why the genetic code is universal and is common to almost all organisms.			X				
SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.					X		
SC.912.L.16.12 Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).		X	X				
SC.912.L 17.11							
SC.912.18.7	X						
SC.912.18.8							
Sc.912. 18.9							
SC.912.N.1.1 Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following: 1. pose questions about the natural world, 2. conduct systematic observations, 3. examine books and other sources of information to see what is already known, 4. review what is known in light of empirical evidence, 5. plan investigations, 6. use tools to gather, analyze, and interpret data, 7. pose answers, explanations, or descriptions of events,		X	X				

Benchmark:	Lesson						
	1	2	3	4	5	6	7
8. generate explanations that explicate or describe natural phenomena (inferences), 9. use appropriate evidence and reasoning to justify these explanations to others, 10. communicate results of scientific investigations, and 11. evaluate the merits of the explanations produced by others.							
SC.912.N.1.4 Identify sources of information and assess their reliability according to the strict standards of scientific investigation.			X				
SC.912.N.1.6 Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.	X			X	X		
SC.912.N.1.7 Recognize the role of creativity in constructing scientific questions, methods and explanations.		X					
SC.912.N.4.2 Weigh the merits of alternative strategies for solving a specific societal problem by comparing a number of different costs and benefits, such as human, economic, and environmental.							
LAFS.SL.2.4	X						
LAFS.1112.L.3.6							

In the field of biotechnology many new techniques and advances have occurred due to the sequencing of the human genome. Current technology is outpacing existing technology especially in genetics. Qualitative genetics is an area that studies non-medallion traits to focus on phenotype changes. Current research is focused on linking phenotypes to applied applications in agriculture, medicine and other industry.

The lessons in this unit provide information on using biotechnology applications in fundamental genomic research. Students will be investigating current technologies in Next Generation Sequencing such as real-time PCR using quantitative models (complex non -mendellian traits), and analyzing gene sequences of *Aradopsis thaliana.* The investigation will help students look into these new technologies being used in genomic research today.

These quantitative traits of study are looking closely at phenotype classes that are not clearly distinguished and variation in in degree rather than kind. The effect of many genes contribute to the phenotypic variation with small additive effects. Variation is caused by both environment playing a -major role in plants and genetics. Phenotype = Genetics + Environment.

The use of genetic markers which are places in the chromosome where individuals are different. The types of markers used in qualitative research are single nucleotide polymorphism or SNP's with is a mutation in a single base variation. This usually the variation between individuals (alleles) involve just a single base A, G. SNP's have the most common form in genetic variation. We will investigate these methods which are QTL analysis and Illuminum Infinnium or single base extension. The technology is leading to whole genome sequencing in the future.

The analysis of these phenotypes are measured with linkage disequilibrium. The SNP's are the genetic markers that we measure to determine any association with the gene and the trait. Linkage disequilibrium is the allele frequency at a specific loci position. As future generations occur, shorter regions on alleles appear due to recombination. These factors during meiotic reproduction include independent assortment and crossing over. If the alleles at 2 loci are not independent of one another they may have some correlation. That is where researchers can use SNP's as genetic markers for studies on what the gene may be controlling. They are looking for genes that control traits. Linkage Disequilibrium is important to gene mapping because it uses tightly linked variants that are strongly correlated. Which is a cost savings for genetic studies.

There are new analysis techniques using these genetic markers to tell which parent contributes what piece of the chromosome. These associations of a trait on a specific locus may be controlling the trait. Quantitative Trait Loci Analysis and Association Genetic Analysis are two current methods that are looking at these quantitative traits.

The goal for agriculture and plant biotechnology is to identify certain breeding crosses to improve or make new products, find diseases and other applications to improve our lives.

KEY QUESTION(S): What is a genetic marker? What is the function of a single nucleotide polymorphism and why is it used to locate mutation?

OVERALL TIME ESTIMATE:
- Advanced Preparation: Teacher may want to give some review on genetic markers and gene expression. Students should have planted the pea genotypes at least 4 weeks in advance of this lesson in the greenhouse or light growing stand.
- Student Procedure: 45-60 minutes

LEARNING STYLES: Visual and auditory

VOCABULARY:

Alleles: Place on a chromosome that contains genes from a parent.

DNA: Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms.

Genotype: The genotype is the genetic makeup of a cell, an organism, or an individual (i.e. the specific allele makeup of the individual). The genotype of an organism is the inherited instructions it carries within its genetic code.

Genetic Markers: A gene or short sequence of DNA to identify a chromosome or locate other genes or specific positons on a chromosome.

Haplotype: Describes the alleles at multiple loci received from each parent.

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Locus (Loci):   A specific location or position of a gene's DNA sequence, on a chromosome.

Phenotype: An organism's observable characteristics or traits. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two.

Segregation of Alleles: Each progeny receives one allele from each parent.

Single Nucleotide Polymorphisms: A variation in a single nucleotide that occurs at a specific position in the genome where each variation is present

LESSON SUMMARY: Students will build background knowledge on investigating quantitative genetics in a reading activity. They will be able to read a real application of phenotypic mutations in genes of a giraffe. They will complete the reading according to reading strategy using individual Lexile levels. They will answer questions from the reading using software from www.Newsela.com. This reading will be the hook for students to investigate current technologies in the use of genetic markers and quantitative genetics to study how genes control certain phenotypic traits. This activity sets the stage for students to perform current technologies of looking at phenotypes using both Mendellian and quantitative traits. They will then use an activity called "What is a SNP" as a virtual lab activity. This lesson will challenge students to look at genetics from a current research point of view utilizing current biotechnological procedures. In this lesson, students will understand the basis of the environmental and genetic factors on phenotypic polymorphisms or variation, and know how we use SNP's as genetic markers for investigation of these changes to an organism.

STUDENT LEARNING OBJECTIVES:
The student will be able to...
1. Describe the difference between a phenotype and genotype.
2. Explain how genetic recombination produces variation in future generations.
3. Define gene markers and single nucleotide polymorphisms.
4. Explain that environment and genetics causes changes in phenotypes.
5. Explain the use of genetic markers in biotechnology.

STANDARDS:
17.10 Analyze factors that influence gene expression. SC.912. L. 16.5, 16.6

17.11 Describe the process of genetic marker assisted selection. LAFS. SL 2.4; SC912. L.16.

34.03 Describe how genetic processes and structures control inheritance in plants. LAFS.1112.SL.2.4, LAFS.1112. L.3.6; SC.912. L.16.1, 2, 4;

34.05 Differentiate phenotypic versus genotypic expression in plant crosses. SC.912. L.16.1, 2, 4;

MATERIALS:

- Teacher access to two websites: http://learn.genetics.utah.edu/content/pharma/snips/. And https://newsela.com/articles/giraffe-genetics/id/17841/
- Students need access to a computer and internet and access to the internet.
- 1 copy of *Student Worksheet: What is a SNP?*
- 1 copy of *Teacher Worksheet: What is a SNP?*

BACKGROUND INFORMATION: Teachers are encouraged to read the student information (four sections:
Teachers are encouraged to read the Teacher Information and Student Assessments.

ADVANCE PREPARATION:

1. Teacher should make a copy for each student for the virtual lab activity: What is a SNP? This can an electronic copy for school districts that have computer access.
2. Students will complete a flipping exercise the night before the activity in Lesson 1. There will be a pre-test to ensure students have background knowledges on phenotypes, genotypes and vocabulary necessary for the unit.
3. Have students complete flipping exercise for homework the night before the unit. Emphasize they need to take notes there will be a pre-test before the lesson. http://learn.genetics.utah.edu/content/variation/sources/
    http://learn.genetics.utah.edu/content/variation/mutation/
    http://learn.genetics.utah.edu/content/variation/outcomes/

Implementation note: For students and classrooms unaccustomed to jigsaws, it can seem a bit confusing and chaotic. Have patience. Collaborative learning experiences are a valuable part of scientific discovery.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (10 minutes) Give students a pre-test on the flipping exercise issued for homework the night before this lesson.

2. (10 minutes) Assign students to read the following article on the Newsela website. Standing tall: Gene analysis finds clues to the giraffe's long neck.
    https://newsela.com/articles/giraffe-genetics/id/17841/

3. (5 minutes) Have them complete the reading and quiz with the appropriate Lexile Level and Quiz and save

4. (5 minutes) Ask the students to share their thoughts on the story. Record their comments/questions on the board or other medium that can be referenced throughout the unit (i.e., flip chart paper, transparency, Smartboard, or computer discussion tools (padlet) etc.)   This is also a good time to assess prior knowledge about genes, evolution and mutation.

5. (5 minutes) Tell the students they are going to have a chance to learn more about how we are using new gene technologies to investigate DNA changes using quantitative genetics.

6. (5 minutes) *Implementation note: Optional stopping point/transition into next assignment using the virtual lab activity on SNP's*
    http://learn.genetics.utah.edu/content/pharma/snips/

*   (5 minutes) Have students complete the summary activity 3-2-1 and turn in to the teacher before class ends. 3 statements about what you learned about SNP's, 2 statements about their applications to research, 1 statement about what something new you learned about using these technologies in disease treatment.

- The teacher will need to review the worksheet and complete a pretest on the unit.

ASSESSMENT SUGGESTIONS:

- Pretest on Flipping Exercise for Lesson 1, Part 1
- Use analytics from the reading activity to check understanding of topic.
- Student worksheet on SNP's can be checked for completion.

EXTENSIONS:

Assign background information as a flipping exercise the night before lesson 1 Part 2:
Have students complete the pretest on these activities when they return for lesson 2.

- (20 minutes) Have students go to the following websites: http://learn.genetics.utah.edu/content/variation/haplotype/ http://learn.genetics.utah.edu/content/pharma/snips/, Have students complete the reading and activity. Handout or use a digital copy of the student worksheet on What is a SNP? Students will turn in their answers to the teacher. They will need to complete the worksheet attached for homework and turn in before Lesson 2.

RESOURCES/REFERENCES:

- Background information: Online Mendelian Inheritance of Peas http://www.jstor.org/stable/2331488
- Background information: https://www.genome.gov/10001665/developing-a-haplotype-map-of-the-human-genome-to-find-genes-related-to-health-and-disease-meeting-summary/
- Background Information: http://csg.sph.umich.edu/abecasis/class/666.03.pdf

- By Washington Post, adapted by Newsela staff
- 05.26.16

1. Please access the article by using this link: https://newsela.com/articles/giraffe-genetics/id/17841/

2. Have students choose the appropriate reading Lexile Level.

3. Students will complete the quiz portion after the reading.

4. Teacher can look at the analytics of the quiz in the binder section of the website

Note: You must sign up and enroll your class to receive analytics.

Genetic variation can be of different types:

1. Discrete variation: Mendel's Peas

2. Continuous Quantitative Variation.
    1. Phenotypic classes are not clearly distinguished.
    2. Variation is in degree rather than kind.
    3. Quantitative variation includes most ecologically and economically important traits in agriculture, forestry, ecology, medicine.

The continuous nature of a quantitative traits it due to he effects of many genes that contribute to the phenotypic variation, with small additive effects, and; Variation introduced by the environment.

Genetic and environmental contribute to phenotypic variation

Components of Phenotypic Variation:

- Quantitative or variation on the phenotypic value (P) can be decomposed into two units, the genotypic value (G) and the environment (E).
- **P = G+E**
- One individual's phenotypic values are the sum of the genes that contribute to the trait variation (G = genotypic value) and the deviation from the environment (E).

What is a haplotype?

Haplotypes are a set of allele values across a set of markers. These markers are called Single Nucleotide Polymorphisms. (SNP's). Organisms with similar haplotypes for a certain region (locus) of DNA are more closely related. There are 3 common haplotypes along with their frequencies in the population. The first SNP has alleles A and G; the second SNP has alleles C and T. The four possible haplotypes for these two SNPs are **AC, AT, GC,** and **GT**. However, only **AC** and **GT** are common; these SNPs are said to be highly associated with each other. (NHGRI, 2001)

Because of the cost of whole genome sequencing it is not yet performed on a regular basis. Using SNP's that are associated with a gene are sequenced and used for research. They can target and test for associations for genes of interest.

In the lessons provided for students they will need to understand that SNP's are used as the genetic markers for sequencing.

We can now analyze and sequence the SNP's associated with a gene of interest and perform quantitative association analysis to techniques to identify the mutations and run further research to see if a gene or gene is associated with the phenotypic traits.

Directions: Based on the information from the flipping exercises from last night, please answer the short answer questions below. Please use complete sentences in your answer.

1. Are genetic mutations constantly occurring in a population? If so, describe what factors are a cause of the mutation.

    _____
    _____
    _____
    _____

2. What are genetic variations?

    _____
    _____
    _____

3. What part of the chromosome regions can mutations occur?

    _____
    _____

4. How are mutations caused by environmental changes? Give one example from the video.

    _____
    _____
    _____

5. How does recombination during reproduction influence variation?

    _____
    _____
    _____

6. Sources of variation include _____ and _____ _____ .

7.   What is a mutation?

    _____
    _____

8. Mutation creates slightly different versions of the same genes, called alleles. What process of reproduction either meiosis or mitosis produces genetic variation? _____

9. **True or False**: Mutations can occur in protein coding regions of the gene and affect the protein function, but it can also cause some change variations in the switches which turn the protein on and off.

10. What other factor besides genetics can cause mutations? _____

NAME: _____   DATE: _____

A.   Guiding Questions

Directions: Use the guiding questions 1-5 below as you investigate information about SNP's from the following website.
http://learn.genetics.utah.edu/content/pharma/snips/

1.   What is a single nucleotide polymorphism?

2.   What is a haplotype? Why do they need to be inherited in pairs?

3.   Why do researchers look for associations between multiple pairs of SNP's?

4.   How do scientists use primer extensions to identify SNPs?

5.   Explain the difference between a genomic approach and a functional approach in identifying and characterizing SNP's.

Name: _____ Date: _____

Directions: Use the guiding questions below answer the questions on the activity on SNP's.

http://learn.genetics.utah.edu/content/pharma/snips/

1. What is a single nucleotide polymorphism?
    SNPs are single-nucleotide substitutions of one base for another that occur in more than one percent of the general population.

2. What is a haplotype? Why do they need to be inherited in pairs?
    Haplotypes are a set of allele values across a set of markers. These markers are called Single Nucleotide Polymorphisms. (SNP's). Organisms with similar haplotypes for a certain region (locus) of DNA are more closely related. There needs to be one pair of alleles from each parent which increases variation.

3. Why do researchers look for associations between multiple pairs of SNP's?
    They need to find some type of association between SNP's to use as a predictive marker for many uses of research including medical care, ecology, agriculture.

4. How do researchers use primer extensions to identify SNP's?
    They add a complementary (cDNA) molecule they call a primer at a SNP position, they then add nucleotides to extend the primer. Nucleotides will only be added to the end of the primer only if the sequence is an exact match. Finally, they compare the lengths of the product using gel electrophoresis.

5. Explain the difference between a genomic approach and a functional approach in identifying and characterizing SNP's.
    A genomic approach sequences the entire genome and is large scale involving hundreds of scientists using computer based analyzation to compare numerous individuals to compare differences. A functional approach is used by a scientist looking for an individual drug or disease response. They compare the genes of one individual to other individuals with or without the disease and compare the SNP's for an individual response.

OVERALL TIME ESTIMATE:
- Advanced Preparation: 30 minutes
- Student Procedure: 30 minutes

LEARNING STYLES: Visual, auditory, and kinesthetic.

VOCABULARY:
Cell Lysis: The breaking down of the membrane of a cell using viral, enzymic or osmotic mechanisms that compromise the cell wall integrity.

Genomic DNA: Deoxyribonucleic acid is a nucleic acid containing genetic instructions used in the development and functioning of all know living organisms.

DNA Isolation: a process of purification of DNA from a sample using a combination of physical and chemical methods.

Elution Buffer: a method to remove the wash buffer using water or Nuclease free water in which is added to the column and filtered through a membrane. The buffer removes the nucleic acid from the membrane and is collected at the bottom of the column.

Lysis Buffer: is a buffer solution used for breaking open cells for used in molecular biology to analyze the compounds of cells using salts to regulate acidity and osmolarity of the lysate.
Washing Buffer

LESSON SUMMARY:
Working in groups, students will review the video clip on Genomic DNA Plant Extraction: They will take notes in the lab notebooks documented the steps needed to extract the DNA from the *Pisium sativium* plants

STUDENT LEARNING OBJECTIVES:
> The student will be able to...
1. Design an agriculture experiment using standard protocol measures.
2. Perform genomic DNA extraction on a plant.
3. Perform purification of DNA in the laboratory.
4. Maintain and interpret biotechnology lab and production records.
5. Demonstrate aseptic techniques during the laboratory procedures.
6. Consider the role technology has played in the rapid advances in biomedical science during the last twenty years.

STANDARDS: See table on page 9-10.

15.02 Design an agricultural experiment using appropriate control measures.

17.09 Perform DNA manipulations, such as cloning/sub cloning, blotting, sequencing and amplification.

18.02 Operate laboratory equipment and measurement devices.

18.01 Maintain and interpret biotechnology laboratory and production records.

18.03 Demonstrate aseptic techniques in the biotechnology laboratory.

18.04 Select an appropriate standard operating procedure for working with biological materials and equipment.

18.05 Prepare buffers, reagents, solutions and media.

18.09 Extract and purify DNA

 LAFS.1112.SL.2.4

 LAFS.1112.L.3.6

MAFS.912.N-Q 1.2

MAFA.912.A-CED.1.3

SC.912.L.16.1

SC.912.L.16.2

SC.912.L.16.4

SC.912.L. 16.11

SC.912.L. 16.12

SC.912.N.1.1

SC.912.N.1.2

SC.912.N.2.4

SC.912.N.2.5

SC.912.P.8.7

SC.912.P.8.11

MATERIALS:

1. *Student Page: DNA Extraction and Purification Protocol*
2. *Teacher Page: DNA Teacher Preparation Document*
3. *Student worksheet*: *DNA Extraction and Purification Lab Sheet*
4. *Teacher Page: Lab Rubric*

BACKGROUND INFORMATION: Background information needed for this assignment is at the beginning of the guide and included on the information cards. Teachers should read the information cards prior to the start of the lesson.

Background information needed for this assignment is at the beginning of the Lab Manual provided for the Extract-N-Amp PCR Kit. Students will be collecting their leaf samples from the selected Mendel pea genotypes grown in the greenhouse. Students will need to collect at least 3 samples from the genotypes to extract and purify.

ADVANCE PREPARATION:

1. Have the students watch the video clips prior to starting the lab. Have them write down the steps in their laboratory notebook and prepare at least one question to ask the instructor as a class discussion. This can be performed as a homework/flipping assignment the night before the lab.
   https://www.youtube.com/watch?v=xlrwef2Y3f0
   https://www.youtube.com/watch?v=iYaxOwZLIAk
   https://www.youtube.com/watch?v=y7kny3Xy4k4
   https://www.youtube.com/watch?v=buzWMKIHbBI

2. Make copies of the lab sheet for each student: DNA Extraction and Purification for students Lab.

3. TEACHER PAGE: Make a copy of the REDExtract-N-Amp Plant PCR Kits Protocol. http://www.sigmaaldrich.com/technical-documents/protocols/biology/redextract-n-amp-plant-pcr-kit.html

Teacher will need to prepare ice buckets, heat blocks or water bath, paper punch or P-1000 micropipeter tips for leaf sample, pea plants from greenhouse, access to freezer for plant material and primers, vortex, micro centrifuge, P-20, P-100 micropipeter and tips, RNASE free water, forceps, PCR Primers, PCR reagent.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (5 minutes) After students have completed the video clips on DNA extraction and PCR from the previous night. Have them answer the 2 Pre-Lab Questions on the Student Laboratory Sheet.
2. (35 minutes) Have student complete the DNA Extraction and store samples in the refrigerator overnight at 2-8⁰ C.
   Students should complete their lab report as they complete the procedures or record in a lab notebook they have used in class. They will need to set up a chart to record the number of their samples along with the genetic code for each plant sample.
3. (5 minutes) Have students stop and clean up the lab stations, dispose of materials according to lab protocols, put DNA samples in the refrigerator overnight in preparation for PCR lab.

Implementation Note: This is a stopping point for this lab procedure, the teacher can decide if they want students to perform the PCR immediately after if they have a block (90 minute) class.

ASSESSMENT SUGGESTIONS:
   The teacher should use the lab rubric attached to grade the lab reports. The lab should be graded after the PCR is completed in Lesson 3.

EXTENSIONS:

Have students pre-read the PCR Lab extensions before they start Lesson 3. http://www.jove.com/video/3998/polymerase-chain-reaction-basic-protocol-plus-troubleshooting

ACTIVITIES:

RESOURCES/REFERENCES:

- Background information: http://www.sigmaaldrich.com/technical-documents/protocols/biology/redextract-n-amp-plant-pcr-kit.html
- Background information:
  http://download.springer.com/static/pdf/927/art%253A10.1007%252FBF03195661.pdf?originUrl=http%3A%2F%2Flink.springer.com%2Farticle%2F10.1007%2FBF03195661&token2=exp=1468352501~acl=%2Fstatic%2Fpdf%2F927%2Fart%25253A10.1007%25252FBF03195661.pdf%3ForiginUrl%3Dhttp%253A%252F%252Flink.springer.com%252Farticle%252F10.1007%252FBF03195661*~hmac=068fc2ae8c3e3c57de126916a05a8d861836303450b2a493bdec4ce76c9e46f4
- Background information: http://biotechlearn.org.nz/themes/dna_lab/dna_extraction

Teachers will need to download the lab protocol for the DNA/PCR lab procedure for the REDExtract-N-Amp DNA Kit.

https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/xnaprbul.pdf

The lab procedures for genomic plant DNA extraction can vary according to the protocols that are chosen and the type of kit needed for the genotype selection. DNA purification may need to be added if you want to sequence the DNA product.

To extract DNA from plant tissue requires a 5 – 6 step process. Many teachers are familiar with classroom DNA extraction using strawberries or other fruit using salt, detergent and alcohol and coming out with many pieces of DNA. In research, DNA needs to have small pieces to be analyzed. A more comprehensive process of extraction needs to be used to be able to run a PCR and look at the genotype of the plant tissue selected.

A basic genomic DNA extraction is used as a routine procedure to isolate DNA from the nucleus of the cells.

Step 1: Breaking cells open (lyse) to release the DNA. This is accomplished by grinding with a mortar and pestle, metallic beads and a vortex and by adding liquid nitrogen or a salt (extraction) solution.

Step 2: Separating the DNA from proteins and other cellular debris. A protease (protein enzyme) is added to degrade the protein. The cellular debris can be removed by using filtration in a column tube.

Step 3: Precipitating the DNA with an alcohol. Ice cold Isopropanol or ethanol is commonly used and added to the DNA sample. DNA is insoluble in alcohol and a salt and forms a precipitate.

Step 4: Removing the RNA by adding RNase

Step 5: DNA Purification (wash) by using an alkaline buffer.

Step 6: An optical density reading can be taken using a spectrophotometer and gel electrophoresis to determine the concentration and purity of the DNA.

After DNA Purification is complete the sample is then amplified using polymerase chain reaction.

DNA Extraction Protocol

Note: 1% Agarose gels should be made by each student group.

Directions:

a. Weigh and measure 1.0 gram of Agarose powder with weighing paper and a digital scale.
b. Add to 100 ml of water and mix.
c. Microwave for about 2 minutes, checking every 30 seconds, until mixture is clear.
d. Cool and poor into gel electrophoresis unit and add appropriate comb for wells.
e. Let solidify and slowly remove gel comb.
f. Let sit and complete the following PCR Steps.

2. With a partner, gather the following lab materials and bring to your lab station.
- Paper Punch (or) P-1000 micropipette tip.
- Forceps (small to medium)
- Heat block or water bath at 95⁰C
- PCR Primers
- Water, PCR Reagant (W1754)
- Collection tube
- P-100 micropipetter
- Beaker for tips

3. **Procedures**:
a. Rinse the paper punch in 70 percent ethanol prior to use or use a clean P-1000 micropipette tip.
b. Punch a 0.5 to 0.7 cm disk of leaf tissue from one pea plant sample. You will punch 3 samples as your replicate.
c. Label collection µtubes 1-1, 2-1, 3-1 etc.
d. Punch a 0.5 to 0.7 disk of leaf tissue for your second plant genotype. Label this 2-1, 2-2, 2-3. Make sure to label the side and cap of the collection tube. Record this information on the data chart in your lab manual along with the genotype of each sample.
e. Add 100 µl of Extraction Solution to the collection tube. Close the tube and vortex briefly. Make sure the disk is covered by extraction Solution.
f. Incubate in a heat block or water bath at 95⁰C for 10 minutes. The leaf tissues usually don't appear to be degraded after this treatment.

g. Add 100µl of Dilution Solution and vortex to mix.
h. Store the diluted leaf extract at 2-8°C. (You can leave the leaf disk in the storage solution)
i. Record all the information in your lab report.
j. Clean up your lab station and dispose of tips as per lab protocols.

PCR Procedure: Part 2

Materials

Reagent	Volume
Water, PCR reagent	x µl
REDExtract-N-Amp PCR Ready Mix	10 µl
Forward primer	y µl
Reverse Primer	y µl
Leaf disk extract	4 µl
Total volume =	20 µl

1. Add the following reagents from the chart above to a thin walled PCR micro centrifuge tube.
2. Mix gently and briefly centrifuge to collect all components at the bottom of the tube. Add the water first and then the other reagents.
3. If your thermal cycle doesn't have a heated lid, add 20µl of mineral oil to the top of each tube to prevent evaporation.
4. The amplification parameters should be optimized for individual primers, template and thermocycler.

Common cycling parameters

Step	Temperature	Time	Cycles
Initial Denaturation	94 °C	3 min.	1
Denaturation	94 °C	0.5-1 min	30-35
Annealing	45 to 68 °C	0.5-1 min	30-35
Extension	72 °C	1-2 min (~1 kb/min)	30-35
Final Extension	72 °C	10 min.	1
Hold	4 °C	Indefinitely	

5. The amplified DNA can be directly loaded onto an agarose gel after the PCR is completed. It is not necessary to add a separate loading buffer/tracking dye.
6. PCR Purification can be purified using the PCR Clean-Up Kit NA1020.(Ask Instructor)
7. The PCR can be run overnight. Stain the gel with Fast Blue if necessary if you don't have a
8. UV illuminator.
9. Clean up your lab station and dispose of materials per lab protocol.

Scientific Method Laboratory Report

NAME: _____

DATE: _____

LAB Title: _____

Purpose or Problem – What is your experiment about or what question are you trying to answer?

_____
_____
_____

Hypothesis (IF Applicable)

_____

Materials

_____

_____

_____

Procedures (Part 1 and Part 2)

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____

Results and Data Collection – (In the form of a table, include drawings or photos).

DATA must include quantitative and qualitative data. Make sure to include a photo of your DNA and PCR results. Please represent the # of base pairs of the PCR on the photo, also label your gel results.   Report your results of the quality of your bands.

Conclusion: What happened? What were the results of the DNA extraction and PCR. Did you have to repeat the experimentation?

_____
_____
_____
_____

Is there anything you would change? What were your sources of error?

_____

_____

_____

STUDENT AND TEACHER PAGE: Lab Rubric

	Excellent 4 pts	Good 3 pts	Emerging 2 pts	Incomplete or Unsatisfactory 1 pts	
Observation/Problem/Question	Excellent When faced with using the scientific method to solve a problem you clearly observed the situation and researched possible solutions.	Good Observations and ideas used to explore possible solutions are not written down or are hard to understand.	Emerging Incomplete. Good intentions, but this aspect of your lab lacks focus. Writing needs work.	Incomplete or Unsatisfactory This part of the report is missing or doesn't provide enough learning evidence.	
Hypothesis	Excellent Is stated as an if...then ... because ... statement. Is directly related to the problem with important supporting details about what may be causing the problem or what could be done to solve it.	Good Is stated in if...then... because ... format but needs to relate to the problem more directly and identify a realistic "because" statement.	Emerging Needs to be stated as if...then... because ... statement. Needs to relate to the problem more directly. Needs supporting detail (a better because).	Incomplete or Unsatisfactory This part of the report is missing or doesn't provide enough learning evidence.	
Materials list	Excellent List is complete for an accurate replication of the experiment. List includes quantitative measurements using the metric system.	Good List is complete but quantities not always there or missing completely.	Emerging Listed but not complete. Quantities missing or are rarely there or the metric system isn't used for data collection.	Incomplete or Unsatisfactory This part of the report is missing or doesn't provide enough learning evidence.	

Reliability/Validity	Excellent	Good	Emerging	Incomplete or Unsatisfactory
	Thought is clearly given to setting up procedures that promote reliable and valid results. A control group used when applicable.	Could have done more to establish reliability or validity by identifying more control variables or other impacts that would change results caused by manipulated variables.	Reliability, validity, controls and manipulatives are mentioned but not implemented. Procedure likely does not test what you think it is testing.	This part of the report is missing or doesn't provide enough learning evidence.
Procedures	Excellent	Good	Emerging	Incomplete or Unsatisfactory
	Written as though giving directions. Written in complete sentences. Are complete with no steps missing. A model or diagram is used as needed.	Logical sequence but missing steps. Some incomplete sentences. The model or diagram that is used is incomplete or distracting.	Needs more steps for the lab to be reliable. Needs complete sentences. Model isn't used when it would have been an obvious addition.	This part of the report is missing or doesn't provide enough learning evidence.
Test Results/Data	Excellent	Good	Emerging	Incomplete or Unsatisfactory
	An accurate data chart and graph is present. The data graph is properly set up and is supported by the data in the chart. Includes relevant qualitative data and other observations.	Data chart needs to be properly labeled to make sense of the data and /or graph needs to be properly labeled to make better sense of the results.	Data missing from the data chart and or graph.	This part of the report is missing or doesn't provide enough learning evidence.
Conclusion	Excellent	Good	Emerging	Incomplete or Unsatisfactory
	Conclusion sums up the data and analyzes how	Some questions about the data are not	Hypothesis needs to be 	This part of the

	the test procedures could have been improved and how the data relates to the hypothesis.	discussed or have incomplete answers conclusions.	restated and data used to describe how it is or is not supported needs more analysis.	report is missing or doesn't provide enough learning evidence.	

KEY QUESTION(S): How do you use bioinformatics to find information on genetic sequencing?
How has this technology helped advance the field of genetic research?

OVERALL TIME ESTIMATE:
2 Part Lesson

- Teacher: 20 Minute introduction to Association Genetics and Sequencing
- Student Procedure: 30-minute Practice Activity using Genetic Mapping.
- Student Procedure: 30 minutes to analyze the sequence using the CLUSTALW sequencing database.
- Student Procedure: 10 minutes to write down results and analyze which mutation and find the genetic markers.
- Student Procedure: 10 minutes for class discussion on sequencing.

LEARNING STYLES: Visual and auditory

VOCABULARY:
Association genetics: analyzing close associations of small pieces of chromosomes that have a close association with a certain trait.

Crossing over: an interchange of genes or segments between homologous chromosome

Phenotype: the observable properties of an organism representing physical traits.

Recombination: the processes of crossing-over and independent assortment of new combinations of genes in offspring that did not occur in the parents

Linkage map: a representation of the relative positions of genes in a chromosome

LESSON SUMMARY: Using Punnett squares, linkage maps and genetic sequencing data bases students will analyze pieces of pea sequences and look at the SNP's and variations between the sequences.

STUDENT LEARNING OBJECTIVES:
>   The student will be able to...
* understand why Mendel's laws of inheritance do not apply to linked genes.
* understand the linear arrangement of genes along a chromosome.
* understand how meiosis and crossing over results in recombinant gametes.
* use web-based resources to find information on genetics to compare close associations of linked genes.

STANDARDS:

24.08 Use web-based resources to find information on the genetic sequence of a protein using bioinformatics. LAFS.1112.RI.3.7; SC.912. L.18.4

34.03 Describe how genetic processes and structures control inheritance in plants. LAFS.1112.SL.2.4, LAFS.1112. L.3.6; SC.912. L.16.1, 2, 4;

34.05 Differentiate phenotypic versus genotypic expression in plant crosses. SC.912. L.16.1, 2, 4

MATERIALS:

* Computer and access to internet
* Copies of theStudent Page: How to make a linkage map
* Copies of the Student Page: Bioinformatics

BACKGROUND INFORMATION: In this lesson students will be analyzing sequences obtained from the UF Forestry Genomics Lab from the PCR sequences that were analyzed. They will complete a background tutorial on association genetics and linkage disequilibrium. Students will use a bioinformatics program to analyze the sequences and find the SNP's. They will then discover the variant traits in their samples.

ADVANCE PREPARATION:
The teacher should have students review the PowerPoint information on linkage disequilibrium and association genetics . make copies of both Student Worksheets

IMPLEMENTATION NOTES:

The worksheet for the linkage map is a review on phenotype dihybrid crosses. Linkage mapping is a new concept. Select portions of the worksheet and activity that is appropriate for the grade level.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATIONS:

Part 1

1. Warm Up Activity: Handout the Linkage Map The students will complete a dihybrid cross using a Punnett square as a review on Mendellian traits. Open up the Worksheet using this link http://www.learnnc.org/lp/editions/american-chestnut/6965

2. Students will then complete the Linkage Map Worksheet demonstrating how some traits are linked (45) (Kenan Fellows Program) You can have the students finish just part of the exercise but have them complete the math portion.

3. Explain to students that two genes are said to be **linked** if they are located on the same chromosome. Different chromosomes segregate independently during meiosis. Therefore, for two genes located at different chromosomes, we may assume that their alleles also segregate independently. The chance that an allele at one locus co-inherits with an allele at another locus of the same parental origin is then 0.5 and such genes are unlinked.   They are uncorrelated. This process is called **linkage disequilibrium.**

4. Ticket Out the Door Summary (5 minutes). Students should answer the essential question at the end of both activities on sequencing.

Part 2: Lesson 3   (50 minutes)

1. Expose the Students to a basic introduction on bioinformatics. Using the Student Page: Analyzing a Genomic Sequence.
2. Students will then take the sequence they received from the UF Lab of their DNA and paste into http://useast.ensembl.org/index.html website to analyze their sequence. from the lab. (30 minutes)
5. Students will design a PowerPoint presentation on what they discovered about their sequences.

ASSESSMENT SUGGESTIONS:

Grade Student Worksheet on linkage mapping

Grade Bioinformatics Worksheet

RESOURCES/REFERENCES:

http://www.plantgdb.org/tutorial/GSannotation/

http://www.umich.edu/~pwlab/Gene%20mapping%20activity.pdf

http://www.learnnc.org/lp/editions/american-chestnut/6965

http://www.animalgenome.org/edu/QTL/Julius_notes/05_linkagemap.PDF

http://www.bio-rad.com/webroot/web/pdf/lsr/literature/10011398A.pdf

NAME: _____   DATE: _____

Directions:

1. Please go the following website and complete the dihybrid Punnett square.
    http://www.learnnc.org/lp/editions/american-chestnut/6965 .

2. Go to the middle of the page and open up the Microsoft Word Document titled linkage map worksheet.

3. Complete the three linkage crosses and draw out the chromosome positions. You can do this in groups and figure out how to calculate the frequencies: Work in groups of two to three and calculate the frequencies.

4. Draw the linear maps of the three chromosomes according to the instructions on the worksheet.

5. When the teacher calls time answer this question.

6. Why is it that some traits are linked? What would cause some traits not to be linked?

7. If some traits are not linked would there be a way to determine if the location of the allele (variation) is being controlled by a nearby gene?

STUDENT PRACTICE: WE WILL BE USING BIOINFORMATICS AS A TOOL TO ANALYZE OUR SEQUENCES. PLEASE GO THROUGH THIS EXAMPLE FOR HOW TO PASTE YOUR SEQUENCE INTO A DATABASE AND FIND RESULTS.

Directions: Go to this website

http://www.ebi.ac.uk/training/online/course/ensembl-browsing-chordate-genomes/guided-examples-using-ensembl/using-sequence-find-gene-blas

1. Complete the tutorial on how to use a bioinformatics data base. Follow the instructions on the screen starting with Step 1.
2. Copy the Sequence and go to Step 2. It should look like the image below.
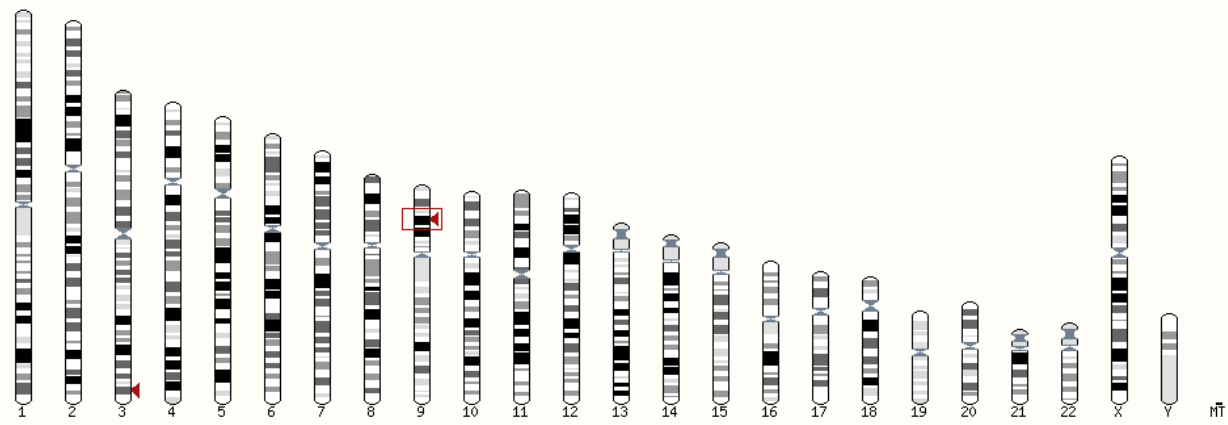

Image Shows a hit on Chromosome #9

3. Go to Step 3: and complete the exercise. You can go through the entire tutorial on the left for more practice.

Part 2: ACTUAL GENE SEQUENCING

Directions: We will receive our sequences from our pea plant crosses we performed at the beginning of the unit. You will not sequence the information into a genetic database. Please follow these instructions.

1.   Receive the sequence from your teacher.

2.   Got the to the following website: http://useast.ensembl.org/index.html

3.   Follow the directions on the page to insert your sequence.

4.   Cut and paste the sequence into the box on the program and get your results.

5.   You should see your sequences in A-T, or G-C sequences (EXON and INTRON)

6.   . Print this page out and find where the SNPs are by looking at the letter substitutions.

QUESTIONS

7.   What is the red code that you see?

8.   What can we conclude from these sequences?

9. How many variations did you observe?

10. Why would these sequences be helpful in looking at the associations between a gene and the way it would control a trait?

KEY QUESTION(S): How can you apply the quantitative genetic research methods to a real world agriculture application?

OVERALL TIME ESTIMATE:
- Experimentation: 8 weeks
- Report : 1 week
- Application: 1 week

LEARNING STYLES: Visual and kinesthetic.

LESSON SUMMARY: Student will choose to do an agriculture science research paper. They will investigate a specific disease in a plant or animal and apply current biotechnology methods to help identify the disease using genetic mapping technology. They can do an experiment and turn the project into an Agriscience Fair project.

STANDARDS: See table on page 9-10.
SC.912.L.14.6
SC.912.L.16.2
SC.912.L.16.3
SC.912.L.16.4
SC.912.L.16.5
SC.912.L.16.9
SC.912.L.18.1
SC.912.L.18.4
SC.912.L.18.11
SC.912.N.1.6
SC.912.N.3.5

Scientific papers can be daunting, full of details and language that is unfamiliar. Scientific papers are best read and considered in small, manageable pieces. Unless you plan to repeat the experiment, you really just need to get the general idea of the questions and answers along with the big idea of the paper. As you become more comfortable with reading journal articles, you will naturally read for more depth and content. When starting out however, the key is knowing what to read, what to skim, and what to skip. Yes. There are parts of a paper that you can skip.

The paper is divided into sections, based generally on the scientific method. Most research papers contain the following sections: Abstract, Introduction, Methods/Materials, Results, Discussion, sometimes Conclusions, and References.

The **Abstract** provides a nice summary of the paper. It might have some unknown words or numbers, but it gives the overall flavor of the paper. It should be read and then re-read at the end.

The **Introduction** gives a history of the topic and discusses what others have found. It also poses the research question(s).

Methods and Materials are most meaningful to those in the field who might want to repeat the research or to help clarify results. Skip this section, but note that as you become more experienced with reading primary sources, it can be helpful to return to this section to better understand some of the results and discussion.

Results are just that. There is no discussion or explanation. They are worth a glance, particularly if any tables are included that summarize the findings neatly. Just a skim of this section will suffice.

The **Discussion/Conclusion** is where the author explains what happened. In this section, the questions should be answered. This is usually where the author reflects on the work and its meaning in relation to other findings and to the field in general.

Re-read the Abstract. Does it make more sense now? It should tie everything together.

Vocabulary. You may need to look words up if you can't figure them out using context clues. You can miss a really important point of the paper if you don't understand the language.

In summary:

- Absolutely read the Abstract, Introduction, Discussion, and then the Abstract again.
- Skim the results.
- Skip the methods/materials.

In the end, you want to be able to answer the following questions with some confidence:

- What was the purpose of the study?
- What questions were asked?
- What were the final answers?
- What was unique about the study?
- What is the next step?

Name: _____

Paper name: _____

1. What was the purpose of the study?

2. What questions were asked?

3. What were the final answers?

4. What was unique about the study?

5. What is the next step?

Student name: _____   Date: _____

Circle One: Pre-test   Post-test

BEYOND MENDEL SUMMATIVE UNIT EXAM

Please answer the questions completely based on what you have learned throughout the unit. Please back your responses up with examples from the laboratories or activities. Please use a separate sheet of paper.

Question #1: What is the difference between genotype and phenotype? Why did we use Mendel's peas to look at the associations of phenotypic changes in the peas? (10 points)

Question # 2: What are basics steps in DNA extraction. Why do we need to purify the DNA before we run a PCR? (5 points)

Question 3: Mendel looked at multiple generations of pea plants with his 7 traits. Using qualitative genetics and genetic markers what can we build on as scientists with looking closer at phenotypic traits and the associations to genes? What has this new technology helped us achieve in medicine, agriculture and environmental applications? List at least one application in each of the areas listed above. (10 points)

Question 4: What does the title "Beyond Mendel" explain about how far we have come in genomic research and new technologies that must be created for us to advance science further? (15 points)