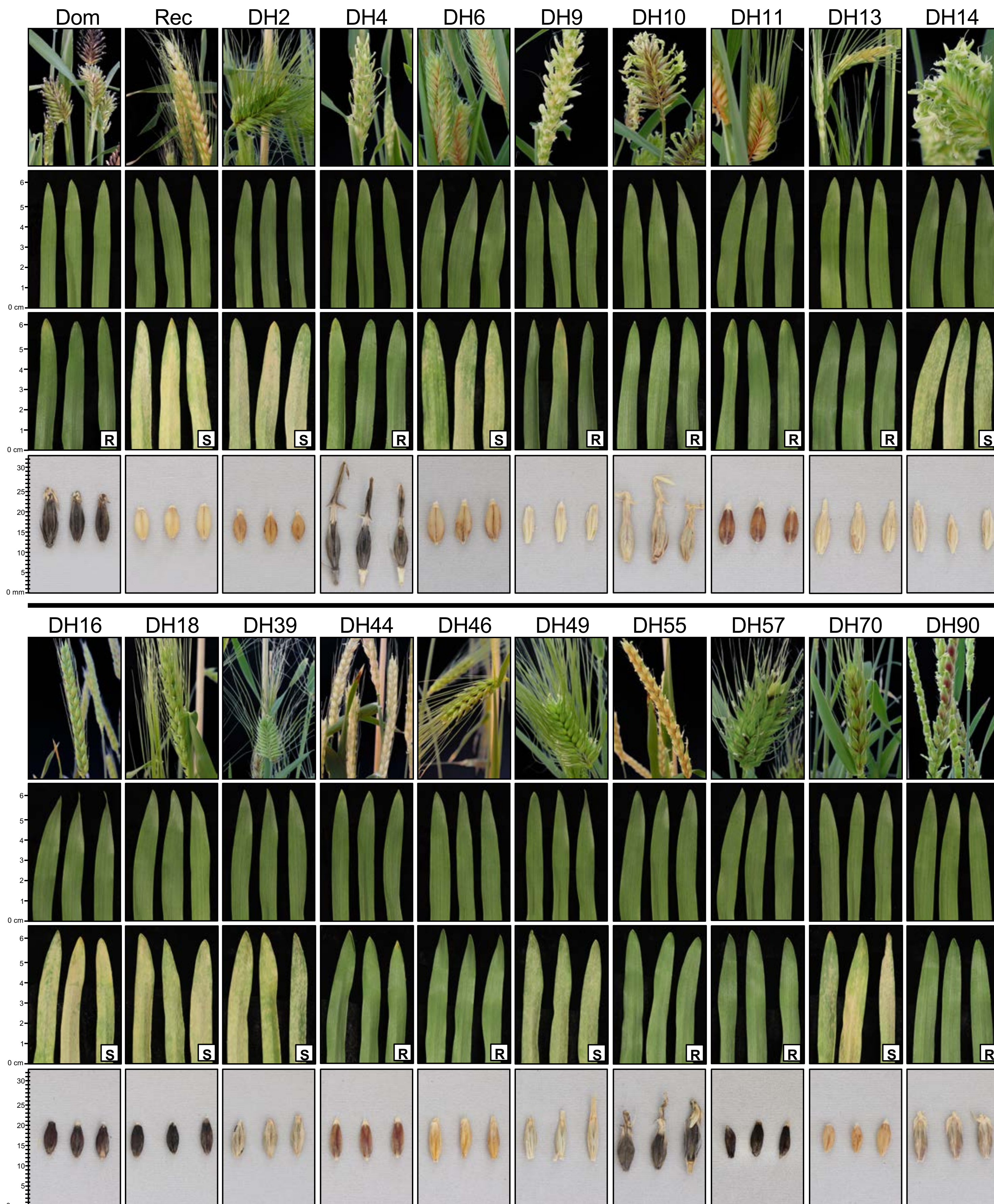
# iTAG Barley: Interactive laboratory exercises designed to explore variations in genotype and phenotype using Oregon Wolfe barley



# "Inheritance of Traits and Genes"

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Additional information and materials can be found at Barley World: https://barleyworld.org/

**Introduction:**

One of the basic concepts in biology is that an organism's physical traits are controlled by its genes that are encoded in DNA. In other words, one's **genotype** for a particular trait controls the **phenotype** that is expressed. Students and novice researchers often struggle understanding this connection between DNA and physical characteristic. 'Inheritance of Traits and Genes in Barley' (iTAG Barley) is a module of laboratory and classroom activities designed to connect visible traits (phenotype) to identifiable differences in DNA sequence of genes.

In this workbook, we will focus on three traits to illustrate basic concepts in plant development, domestication, and disease resistance. Students plant and grow barley plants so that phenotypic variation can be observed first hand. For the first learning module, students will describe the **"awned**" and **"hooded"** phenotypes and use common biotechnology methods to investigate the differences in the DNA sequence at one gene (*Kap*) that influences the development of barley spikelets (**Figure 1**). Students completing this module will learn basic molecular biology techniques of DNA Extraction, Polymerase Chain Reaction**,** and Gel Electrophoresis and interpret data from different plant phenotypes to document **DNA polymorphisms** among plants with different phenotypes.

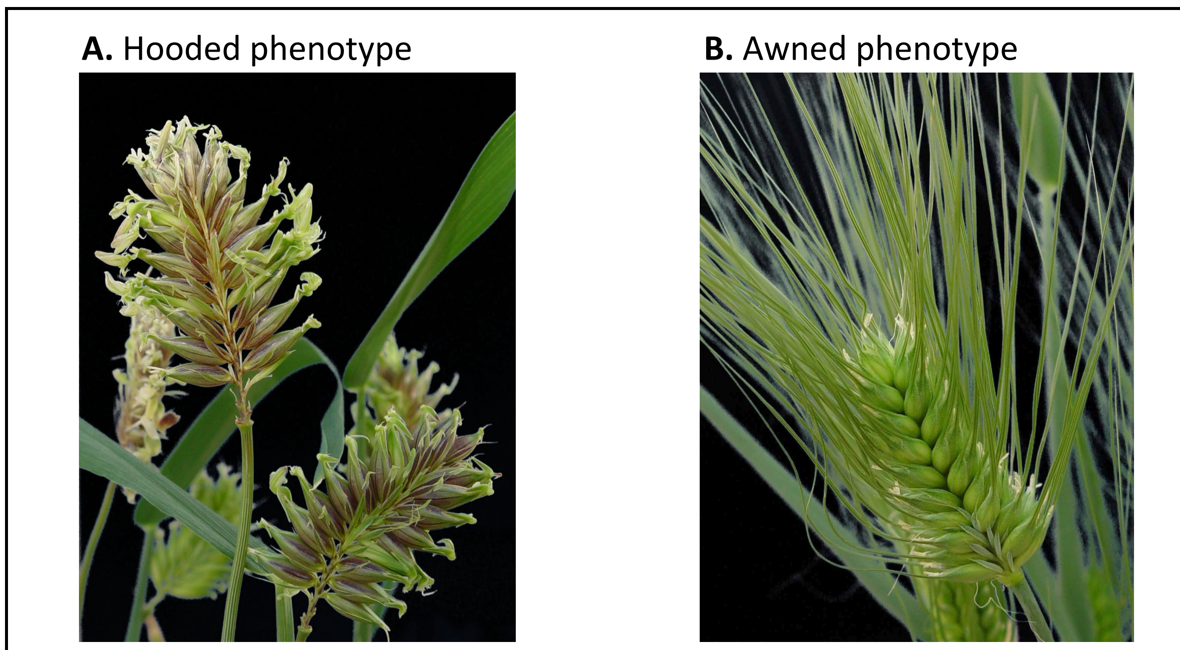


**Figure 1.** Comparison of the Hooded (Panel A, DH10) and Awned (Panel B, DH49) phenotype in Oregon Wolfe Barley. These phenotypes are controlled by the interaction of two genes, *Kap* (Müller *et al.* 1995, Roig *et al.* 2004) and *Lks2* (Yuo *et al.* 2012), respectively.

**Why Oregon Wolfe Barley?**

The Oregon Wolfe Barley population (OWB) is a model resource for genetics research and instruction. This collection of doubled haploid (DH) lines was developed from an **$F_1$** of a cross between dominant and recessive marker stocks advanced by Dr. Robert Wolfe at Federal Agriculture Research in Alberta, Canada. These DH lines originate from a wide cross and have exceptionally diverse and dramatic phenotypes, making the OWB population attractive for teaching basic plant development, Mendelian and molecular genetics, and genomics in high school, community college or first-year university biology (Cistué *et al.* 2011, Giménez *et al.* 2021, Szűcs *et al.* 2009). Dr. Pat Hayes at Oregon State University further selected the 'Informative and Spectacular Subset (ISS)' from which these lessons are based (Hayes 2011, Hayes 2023a, Hayes 2023c, Hayes and Stein 2003).

Using the iTAG Barley module, students can observe the OWB spikes for **seed-coat color**, **two row** vs. **six row** (encoded by *Vrs1*, a domestication trait where two row is dominant and six row is recessive) (Komatsuda *et al.* 2007), **hooded** vs. non-hooded (*Kap*: dominant allele encoded by *BKn3* - a **homoeotic mutation** where the awn is replaced by a duplicate spikelet) (Müller *et al.* 1995, Roig *et al.* 2004, Williams-Carrier *et al.* 1997), and **long awn** vs. **short awn** traits (long awn is dominant and encoded by *Lks2*) (Yuo *et al.* 2012). Lastly, the OWB population has lines that are resistant or susceptible to powdery mildew disease, due to traits encoded by different alleles of *Mildew locus a* (*Mla*) (Seeholzer *et al.* 2010, Wei *et al.* 2002), *Mla6* and *Mla8* (Bettgenhaeuser *et al.* 2021, Halterman *et al.* 2001). Additional details and resources can be found at: **Oregon Wolfe Barley Data and GrainGenes Tools - An archive and resource hub** https://wheat.pw.usda.gov/ggpages/maps/OWB/ (Hayes 2011)

In a series of three different exercises, students perform the **polymerase chain reaction (PCR)** to amplify the *Kap*, *Vrs1* (*HvHox1*), and *Mla* genes using DNAs they isolate from plants with phenotypic differences, complete gel electrophoresis using agarose gels, and document their results by estimating PCR product size by comparing PCR products with size-markers. They then discuss their results and describe differences in the DNA at each gene locus and differences in whole plant phenotypes in the OWB population. Teachers can then lead a discussion of **cosegregation** and how researchers associate genotype and phenotype.

***Goals: After completing the iTAG Barley module students will:***

- Understand the role of DNA in an organism.
- Understand the relationship between a **genotype** and a **phenotype**, including **homeotic mutations**, **epistatic interactions**, and the impact of phenotype on yield.
- Experience science as it is done in a research laboratory.
- Understand that science takes time.

## Project Overview

Students begin by planting a population of Oregon Wolfe Barley. By placing the responsibility of planting, watering, fertilizing, etc. on the students they develop a vested interest in the plants.

Because one of the goals of this module is for students to understand the relationship between an individual's genotype and phenotype, they start by the **amplification** of a single gene (*Kap*) using **PCR**. The genotype of each plant in the population can be compared to the phenotype to observe **cosegregation** of DNA differences with differences in phenotype. This connects molecular differences to phenotypic outcomes**.** The **primers** utilized to amplify the ***Kap* gene** are found on either side of an intron that may have an insertion (*Kap* allele) or not (*kap* allele). The presence or absence of this insertion allows us to identify the two alleles using **gel electrophoresis**. After running gels, students use **gel Green DNA stain** and ultraviolet transilluminators to visualize bands of DNA. The Gel Green is both non-toxic and light insensitive, making it safe and convenient to use. Once the gel data has been documented, the class will discuss the role of gene interactions (**epistasis**) on the development of a phenotype.

The second project also uses PCR, but this time students amplify the ***Vrs1* gene**. The two alleles we will investigate (*Vrs1* and *vrs1*) differ in sequence, which cannot be detected by PCR alone with the primers we will be using. Students will cut their PCR products with a **restriction enzyme digest** and use electrophoresis to distinguish the two alleles.

The last project of this trio of experiments involves plant pathology. One of the traits we will look at encoded by the *Mla* locus and the resistance or susceptibility to powdery mildew, a fungal disease (Halterman *et al.* 2001, Halterman *et al.* 2003, Halterman and Wise 2004, Halterman and Wise 2006, Seeholzer *et al.* 2010, Wei *et al.* 2002)

## Transfer of Concepts : Genotype to Phenotype

Barley is the experimental organism in this module; however, the concepts can be applied to all plants. In many areas of the country, the economy is largely dependent upon agriculture. Because genes determine traits, discussion of genetic engineering and its influence on agriculture is a simple but meaningful application. Students investigate naturally occurring traits (e.g. hooded vs awned, 2-row and 6-row) that are associated with molecular differences that they can detect by closely examining the DNA at a particular genetic locus. This can be the basis for a discussion genetically modified organisms (GMO), selective breeding and domestication of plants for agriculture.

While agriculture is vital to human civilization, students often struggle with plants as living organisms with heritable traits. Once students have completed the first module (investigation

of the *Kap* gene locus), the teacher can lead a discussion linking the student findings, modeling crosses between plants with awned and hooded spikelets and making predictions. The same principles that plant geneticists used to associate the *Kap* gene with the hooded phenotype are used regularly to associate genes with inherited human conditions such as **Cilantro-taste preference** (Eriksson *et al.* 2012), **Sickle Cell** or **Tay Sachs**.

## Connecting the Central Dogma of Molecular Biology to barley phenotypes

The Central Dogma of Molecular Biology states that information stored in DNA is used as a template in transcription, which forms different types of RNA (**Figure 2**). One type of RNA (mRNA) is used as the template for ribosomes to make proteins. Proteins serve many different functions in a cell. Some are signaling molecules, while others make up the structure of the cell, or catalyze reactions.

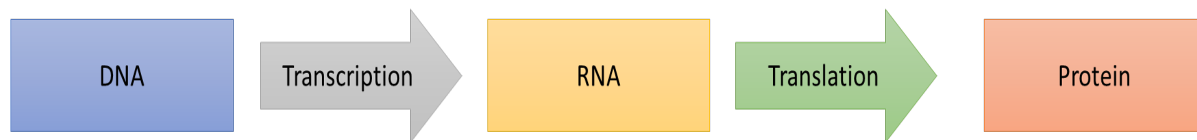


**Figure 2**. Central dogma of molecular biology.

A gene is a functional segment of DNA that encodes a protein (e.g., an enzyme) or RNA molecule (e.g., tRNA). It is the instructions to make its gene product. Since the OWB plants are doubled haploid, they have two copies of every gene (on 2 homologous chromosomes). We call the location of each gene its genetic locus (or location), typically by describing where it is on the chromosome it is found on. In diploid (2N) species (like humans) there are also two copies of each gene locus. The information at a given gene locus is similar for the two chromosomes, but not necessarily identical. These alternate forms of DNA at the same genetic locus are called **alleles**. For example, in peas there is a **locus** for flower color, but this locus may encode a purple (*P*) or white allele (*p*). By convention, we abbreviate the dominant allele with a capital letter (AA or BB) and the recessive with a lower-case letter (aa or bb). The genotype of an organism lists the alleles present at a given locus. For example, AA, Bb, cc are genotype abbreviations for 3 different genes (A, B, C) and the individual in question has 2 copies of the dominant allele for the A locus (AA), one copy of the dominant and one copy of the recessive (Bb), and two copies of the recessive allele (cc). Often the dominant allele encodes a functional protein, so only one copy of this allele is needed to see an observable trait (**phenotype**). The recessive allele often has an error that makes the protein that is produced non-functional, which means both copies of the genetic locus need to encode the recessive allele for it to be observed.

The *Kap* gene and *Lks2* gene loci each encode proteins that influence the production of barley spikes (**Figure 3**). The *Lks* gene locus determines if the barley grain will have a long awn (*Lks/_*)

or short awn (*lks2/lks2*). There is a complicating factor in that plants with the *Kap* allele will form a spikelet instead of an awn, if there is dominant *Lks2* allele present. The recessive *lks2/lks2* plants may have the dominant *Kap*, but will not show it in their phenotype because the *lks2* gene masks the *Kap* phenotype (hooded). We call this interaction **epistasis**, where the gene products from two or more genetic loci will influence a single trait (in this case, the appearance of the barley spikes) (Hayes 2023b).

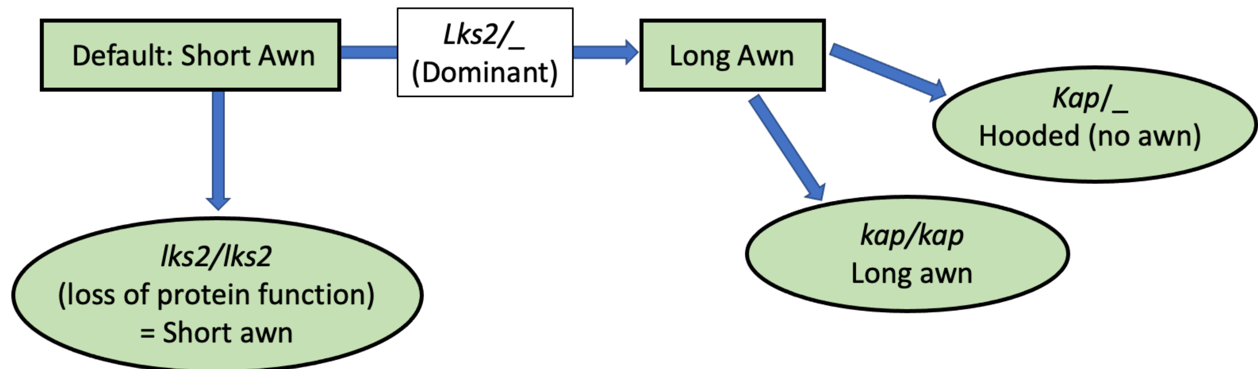


**Figure 3**. *Kap-Lks2* interactions. Plants that carry 2 copies of the recessive allele for *lks2* will not produce a protein needed to make either a long awn or hooded phenotype. This means a plant could have the dominant *Kap* allele (*Kap/_*) and still not show the hooded phenotype if it lacks the dominant *Lks2* allele (*lks2/lks2*).

## Experiment #1 Molecular analysis of the *Kap* genetic Locus

### Overview

In this section you will find the protocols to successfully run the core activities of 'Inheritance of Traits and Genes in Barley' (iTAG Barley). This module can be used in a lecture (50 minute), or lab (90 minute) class period. The module can be made to fit into existing curriculum, or it can also be modified to be shorter or longer (see Extensions to the Module section).

### Learning Objectives: Molecular analysis of the *Kap* genetic locus

**Upon completion of this exercise students will be able to:**

- Differentiate between genes and alleles in terms of genetic loci and observed traits.
- Diagram the process of PCR and explain its value in molecular biology
- Extract DNA from plant tissues and analyze its genotype using PCR.
- Predict the phenotype of plants given gel electrophoresis data from the *Kap* PCR products

### Planting your Oregon Wolf Barley (OWB) seeds

**Materials:**

5-inch Pot
Seed Packet
Fine Point Permanent Marker
Masking Tape
Plant Tag
Standard Potting Mix

**Planting Instructions:**

1. Obtain a pot, marker, tag, tape, and seed packet from instructor.

2. Label your tag with OWB # (from the seed packet label), date, class period, and your name. Label the tape the same way and apply it around the top of the pot. This will be used to identify your plant from others.

3. Fill the pot to the top with soil in a scooping motion, but do not compact the soil into the container.

4. Place your finger into the soil to the first knuckle (~1 inch deep) three times to make three separate spaces for the seeds.

5. Drop one seed in each of the three holes that you created, lightly cover with remaining soil.

6. Place the tag into the soil for easier identification. Place the pot under the light bank (or growing area).

7. Water your plant so that the soil is moist. Make sure the seed does not float to the top.

8. Seedlings should emerge within one week.

## Leaf Tissue DNA Extraction

**Materials:**

2.0 ml Microcentrifuge Tubes
Tube Pestle or Glass Rod
Razor Blade
Vortex
0.3 g of Leaf Tissue
Glass Slide
Gloves
Centrifuge

**Reagents and Buffers:**

2X CTAB Buffer
5M potassium acetate (stored at -20 °C)
70 % ethanol (stored at -20 °C)
2-Mercaptoethanol**
20 % (w/v) sodium dodecyl sulfate (SDS)
Absolute isopropanol (stored at -20 °C)
TE Buffer
Rubbing Alcohol

**Day 1 Harvest Plant Tissue**

1. Label the top and side of a 2.0 ml centrifuge tube with plant number, class period, date, and name using a fine tip permanent marker (e.g., Sharpie).

2. Clean the glass slide, razor, and glass rod with rubbing alcohol to remove any foreign DNA.

3. Collect 3-4 leaves (they should be 3-4 inches long). Find the mass of the leaves until you obtain ~0.3 g leaf tissue.

4. Place leaves on the clean glass slide. Chop the tissue into very small pieces using a clean razor blade. The more finely chopped the better DNA extraction will be.

5. Immediately transfer tissue to the labeled 2.0 ml microcentrifuge tube and further grind tissue with the clean glass rod. Mash the tissue into a wet pulp for 2 minutes.

6. Add 800 µl of **CTAB Buffer** and **2-Mercaptoethanol** solution from the tube labeled CTAB-2Mercap and 100 µl of **SDS** from the tube labeled SDS. Shake by hand, or **Vortex** gently to mix.

7. Place your microcentrifuge tube on ice and give to instructor for overnight storage.

8. Clean Up: clean your slide, razor, and glass rod with rubbing alcohol.

Make sure each tube is properly labeled as the students give them to you. The permanent marker can often get rubbed off.

Store these samples overnight at 4 ˚C or freeze for longer storage.

**** 2-Mercaptoethanol** is toxic and dangerous for the environment. Therefore, it should not be thrown away in the trash. The tubes should be left open under a fume hood in order for the chemical to evaporate from the leaf tissue. Once evaporated, the tubes are safe to throw away in the trash.

The odor of **2-Mercaptoethanol** is similar to the odorant added to natural gas. Vapors can irritate the eyes and mucous membranes. The amount being used in this extraction is minimal and the teacher should be the only person to handle the stock solution in a fume hood.

**Day Two: Extract DNA**

1. Allow your sample to thaw out if necessary. You can speed this up by holding the microcentrifuge tube in your hand. Use the **vortex** to mix up the contents by holding the microcentrifuge tube down on the rubber disc for 15 seconds, or until all of the material is mixed up.

2. Incubate the tube at 65 ˚C for 10 min. Place the tube in the tube holder in the water. Make sure not to disrupt other tubes.

3. While you are waiting, put crushed ice in your cup for the next step and get a tube of cold potassium acetate and tube of cold absolute isopropanol (labeled AI) from the instructor and put on the ice.

4. After the 10 minutes has elapsed, remove your tube from the water bath and place it on your ice.

5. Add 410 µl of cold **potassium acetate**. Mix by inverting the tube up and down 10 times. Place the tube back on ice for 3 minutes.

6. Keep your tube on ice until all groups are ready. Then, bring your tube to the instructor to place in the centrifuge. The tubes will spin at 13,200 rpm for 15 minutes at room temperature. While this is taking place, obtain a new 2.0 ml microcentrifuge tube and label it with the plant #, class period, date, and name (same as the first tube).

7. When the centrifuge has stopped, obtain your tube and return to your lab bench. Transfer approximately 1 ml of the **supernatant** to the new 2.0 ml microcentrifuge tube. This can be tricky as you have to place the pipette tip past the film on top of the liquid and only draw up the liquid above the green plant matter (which should be clumped together at the bottom of the tube). There should not be any green material drawn up into the pipette tip.

   **To ensure you get ~1 ml of clear supernatant you may need to repeat the centrifugation step with the same the tube.**

8. Add 540 µl of ice-cold **absolute isopropanol**. Invert the tube 10 times to mix, and place the tube back on ice for 20 minutes and bring to the instructor for overnight storage. Bring your used tube with plant tissue in it to the instructor for disposal (DO NOT THROW AWAY OR PUT DOWN DRAIN).

**<u>Day Three: Purification of DNA</u>**

1. Allow your sample to thaw out if necessary. You can speed this up by holding the microcentrifuge tube in your hand. Use the **vortex** to mix the contents by holding the microcentrifuge tube down on the rubber disc for 15 seconds, or until all of the material is mixed.

2. Centrifuge at 10,200 rpm for 10 min. Your tube should now contain a clear solution and a small pellet of DNA that is stuck to the wall of the tube, just above the bottom. Discard the **supernatant** by pipetting it out. Be careful not to disturb the pellet.

3. While you are waiting, put crushed ice in your cup and get a tube of 70 % ethanol (labeled E) and a tube of TE Buffer (labeled TE) from the instructor and put on ice.

4. Wash the pellet once with 500 µl **70 % ethanol**. <u>Gently</u> invert tube several times; do not break up the pellet. Pipet the excess ethanol from the tube, again being careful to avoid the disturbing DNA pellet. Let any excess drops on the side of tube dry.

5. Add 200 µl of **TE**. **Vortex** gently to re-suspend the DNA pellet in the TE. Give the tube to your instructor for overnight storage.

### Polymerase Chain Reaction of the *Kap* Gene

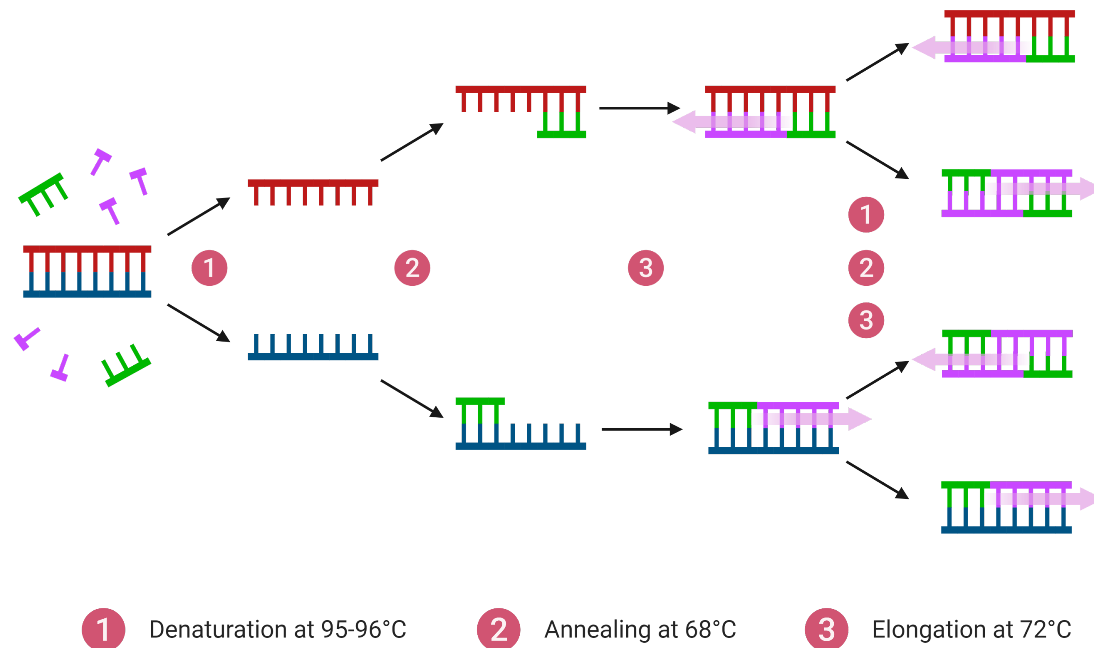


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**Figure 4**. Schematic concept of polymerase chain reaction (PCR).

**Materials:**

| | | |
|---|---|---|
| Thermocycler | 1.5 ml Centrifuge Tubes | Ice |
| Micropipettes | Pipette Tips | *Kap* PCR Primers |
| Molecular Grade Water | DNA Template(s) | |

DNA Taq Polymerase (beads or Master mix)

For PCR: 0.2 mL PCR tubes

1. Obtain your DNA in the 2.0 ml microcentrifuge tube. Begin to thaw it out. Obtain a PCR tube with a ***Taq*** **Polymerase** bead at the bottom and label it like the other tubes with your OWB #, class period, date, and initials (you can just use your initials instead of your full name since the tube is small).

2. While you are waiting, put crushed ice in your cup.

3. Make sure the bead is at the bottom of the tube. Your instructor will add 24 µl of the hooded/awn (*Kap*) primer mix to your PCR tubes.

4. Add 1 µl of your DNA template to your PCR tube.

5. Vortex the tube until the bead fully dissolves and the solution is clear.

6. Tap the PCR tube on the table to get all of the solution down to the bottom of the tube.

7. Store tubes on ice until instructed to transfer your tubes to the **thermocycler**.

8. Give your tube of DNA back to the instructor for storage.

### *Using gel electrophoresis to analyze PCR products*

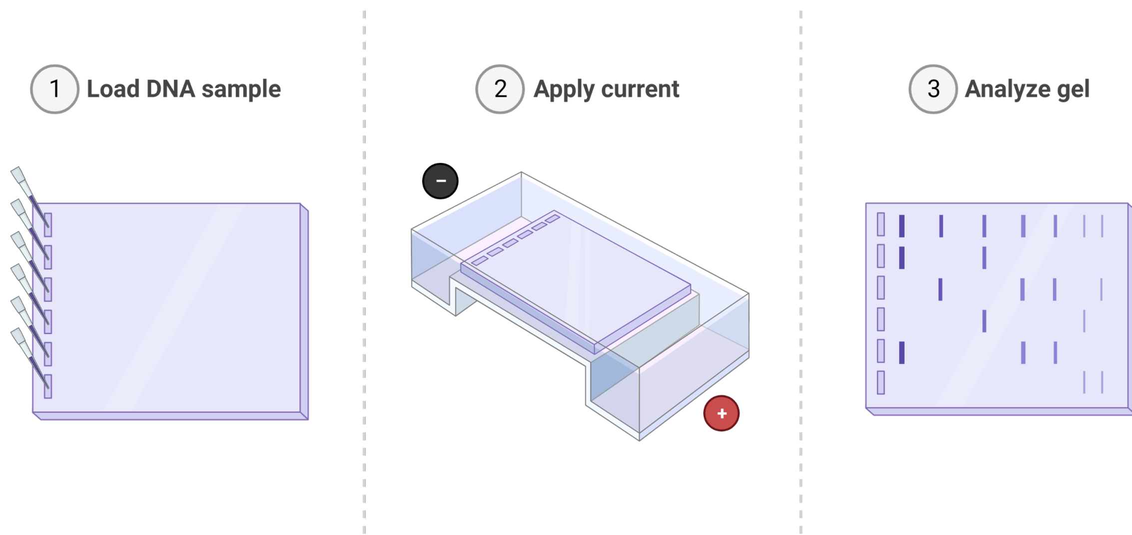


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**Figure 5**. Schematic concept of gel electrophoresis.

**Materials:**

| | | |
|---|---|---|
| **1X TBE Buffer** | 500 ml Flask | Wax Paper or Parafilm |
| Gel Box | **Agarose** | **Gel Loading Dye** |
| Lab Tape | Balance or Scale | Micropipettor and Tips |
| Microwave | Hot Glove | **Gel Green DNA Stain** |

### *Preparing & Loading the DNA*

1. Obtain you tubes containing PCR products from the instructor.

2. On a piece of wax paper or parafilm, combine 3 µl of **loading dye** with 10 µl of PCR product. Draw up and dispense the mixture with your pipette three to four times to mix it. Your product should be a blue color once mixed.

3. Take your wax paper over to the gel electrophoresis. Add 10 µl of the blue DNA/dye mixture to the correct gel well indicated by the paper and your instructor.

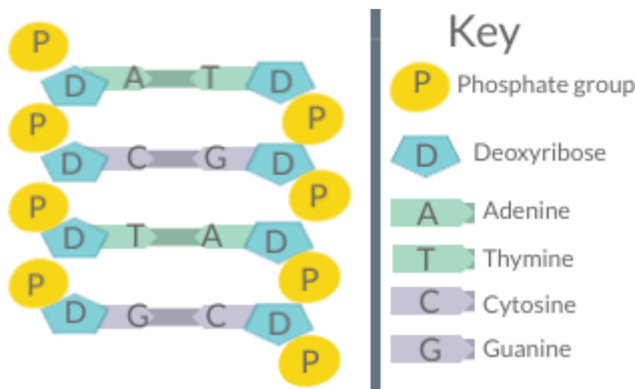
4. Repeat Step 1-3 for each sample you want to load onto the electrophoresis gel. If you are going to be loading numerous samples, it may be helpful to draw a grid on your wax paper to indicate which DNA samples are which.

5. Be sure to use a new tip for each sample! Don't worry about getting the micropipette tip point down into the gel well. Hold the tip over the gel well you are targeting and dispense the DNA. The loading dye causes it to sink down into the well.

When all the samples have been loaded and recorded, place the lid on the gel box. Plug the leads connected to the lid into the power source and turn on the current. Run the gel for 30 minutes at 100 volts. (You (or your teacher) may have to run it longer than 30 min.)

### *How Gel Electrophoresis Works*

Looking at a strand of DNA, we can see that it looks like a twisted ladder, with the "rungs" being the bases (A, T, C, or G), and the sides of the ladder consisting of sugar molecules joined together with phosphate molecules. Those phosphate molecules give the DNA molecule as a whole a negative charge.




Wikimedia commons: by Christinelmiller

Because DNA is negatively charged, it will migrate from a negatively charged anode (black) to a positively charged cathode (red) in an electric current. You can use the mnemonic "run to red" as you put the lid on the electrophoresis chamber. It is important to note, the larger the DNA fragment, the harder it is for the DNA to move around the agarose, so they move more slowly. This means given the same amount of time, the shorter fragments will move further and the larger fragments will stay closer to the wells where you added your sample.

### *Visualizing the Gel:*

1. The DNA is not visible at this point, but during the electrophoresis the Gel Green stain is intercalated into the DNA. In order to see the DNA, gently remove the gel from the gel box or plastic bag/wrap and place it upon the blue platform of the Vernier Transilluminator. Lower the orange lid and turn the light knob. The DNA bands should become visible (**Figure 6**). The darker the surroundings, the better the bands show up. Turning off the room lights room can help.

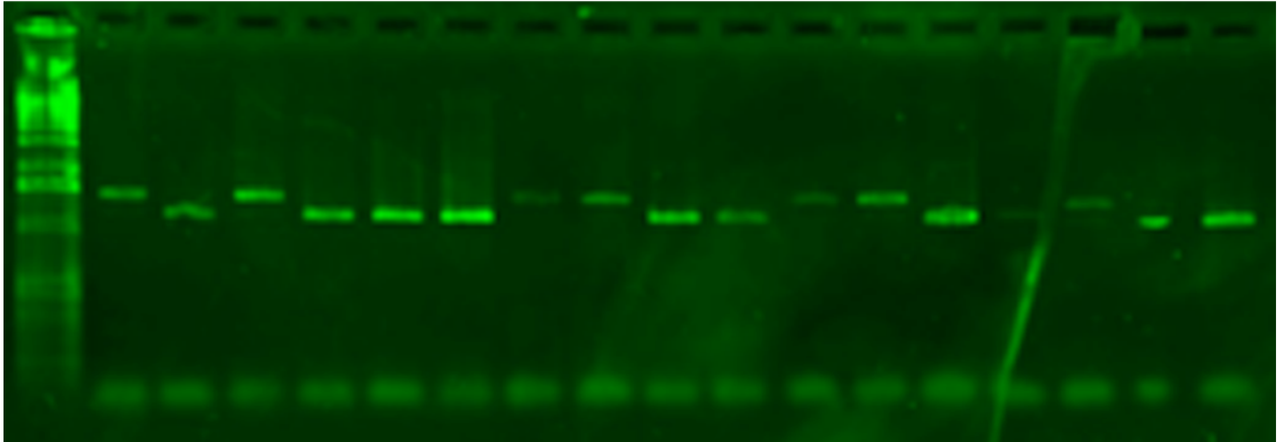


**Figure 6**. DNA stained with Gel Green on the transilluminator.

**Discussion Questions**

1. How are genes and alleles related?
2. What analogy could you use to explain the difference between a gene and an allele?
3. We are purifying DNA from a small segment of the barley plant, how can we be sure this plant tissue has the genes we are investigating?
4. Why do scientists use PCR?
5. What determines how fast a segment of DNA will move through the gel?
6. Would you expect the Kap allele to migrate faster or slower on a gel?
7. Would this type of analysis (PCR and visualization) allow researchers to separate alleles that only differ in DNA sequence, not size? Why or why not?

## Experiment #2 Restriction Length Polymorphisms and the *Vrs1* gene.

### Overview

The *Vrs1* gene has two alleles, one of which codes for a **two-row seed spike** (*Vrs1*) in the adult barley plant, and the other which codes for a **six-row seed spike** (*vrs1*) (Komatsuda *et al.* 2007)**.** The two-row phenotype is dominant over the six-row (**Figure 7**). Unlike the *Kap* and *kap* alleles, the *Vrs1* and *vrs1* differ in terms of **DNA sequence**, not the size of the genetic locus. As shown in Figure 7, the dominant two-row gene encodes a protein that inhibits spike development, leaving the plant with two rows of seeds in the barley spike. The recessive allele (*vrs1*) has a sequence change that causes the protein produced to lose function. This means the lateral seed positions are no longer inhibited, resulting in a six-row barley spike.

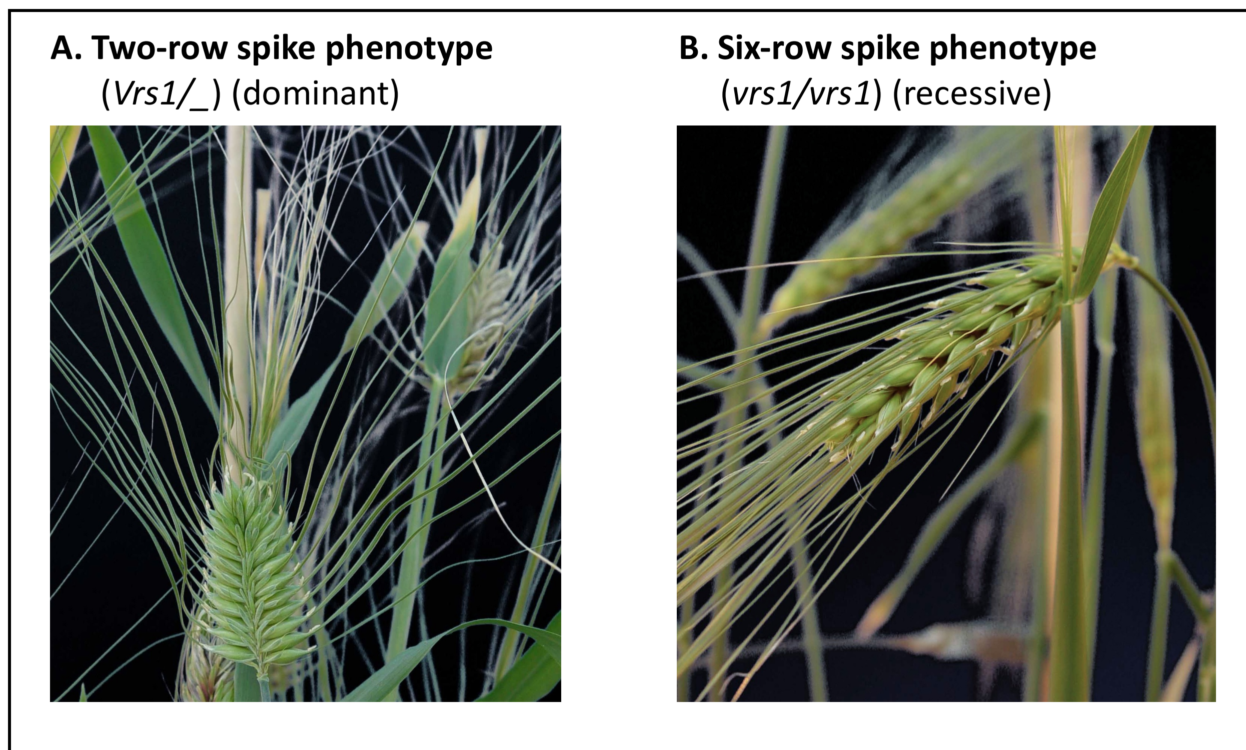



**Figure 7.** Comparison of two-row (Panel A, DH39) and six-row (Panel B, DH46) phenotypes in OWB. These phenotypes are controlled by alternate alleles of the *Vrs1* gene (Komatsuda *et al.* 2007). The six-rowed phenotype originated multiple times and in different regions, through independent mutations of *Vrs1*. The gain in yield established barley as a founder crop for Near Eastern Neolithic civilization.

## Learning Objectives

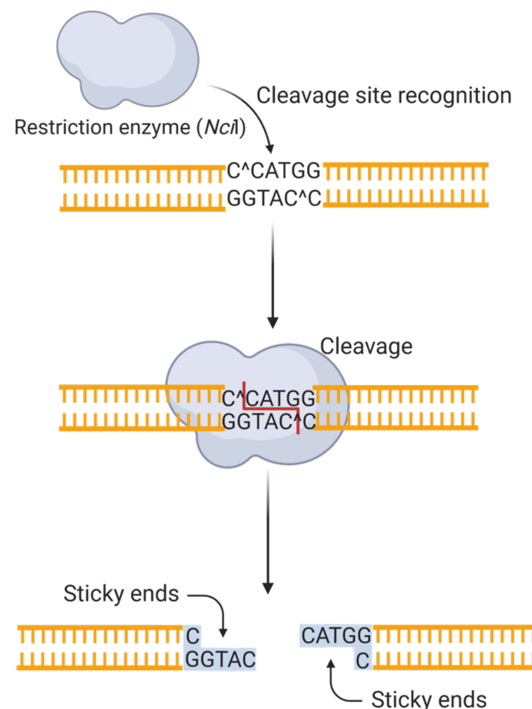
Upon completion of this exercise, students will be able to:

- Explain the limitations of PCR and gel electrophoresis to detect differences in alleles.
- Outline the process of restriction digestion and explain how it can detect DNA sequence changes.
- Interpret gel electrophoresis data to identify allelic differences in the *Vrs1* gene.
- Discuss and evaluate evidence to support claims concerning GMOs and domestication of plants by artificial selection.

## Introduction: Restriction Digests

Since the two alleles are the same length in terms of DNA base pairs, we cannot simply use PCR and gel electrophoresis to differentiate between the 2-row and 6-row plants. In order to determine the genotype at the *Vrs1* locus, we will amplify the plant DNA be using PCR and then digest it with a **restriction enzyme** (**Figure 8**). Restriction enzymes will cut DNA at specific sequences. The enzyme used in this activity, *Nci*I, cleaves the dominant two-row allele in three places, resulting in four DNA bands during electrophoresis. The change in DNA sequence in the *vrs1* (6-row) allele happens to be at one of the *Nci*I cut sites, eliminating this cut-site. So *Nci*I will cut the recessive six-row allele in two places, resulting in only three fragments.

**A. Restriction Enzyme Action**



**B. A mutation in OWB recessive (6-row) deletes a cut site for *Nci*I**

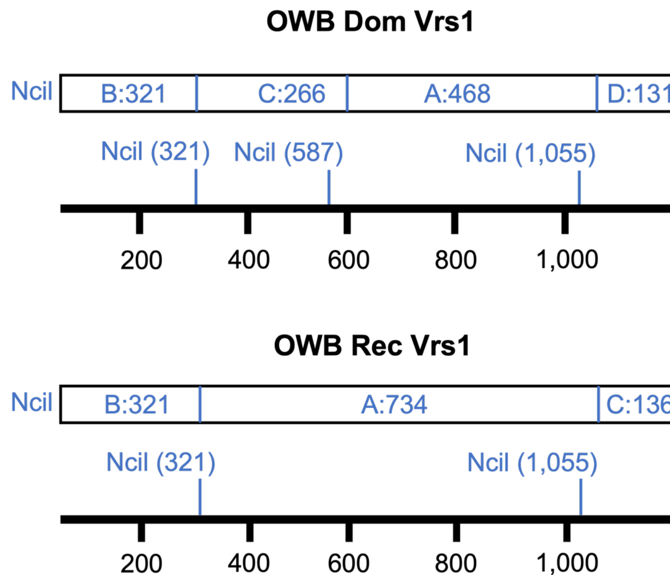



**Figure 8.** A. Restriction Enzymes Cut DNA at specific sequences. If you change the sequence, the cut site is not recognized, so the DNA remains intact. B. Restriction Enzyme Cut Sites for 2-row (Top) and 6-row (Bottom) allele of the *Vrs1* locus.

## Polymerase Chain Reaction of the *Vrs1* Gene

**<u>Materials:</u>**

| | | |
|---|---|---|
| Thermocycler | 1.5 ml Centrifuge Tubes | Ice |
| Micropipettes | Pipette Tips | *Vrs1* PCR Primers |
| Molecular Grade Water | DNA Template(s) | |

Regular PCR Tubes Protocol: 0.2 ml PCR Tubes, Taq DNA Polymerase, & Master Mix

PCR Tubes with Taq Beads Protocol: 0.2 ml PCR Tubes with Taq DNA Polymerase Beads

### *PCR of Vrs1 gene from 2-row and 6-row samples*

1. Obtain your DNA in the 2.0 ml microcentrifuge tube. Begin to thaw it out. Obtain a PCR tube with a ***Taq* Polymerase** bead at the bottom and label it like the other tubes with your OWB #, class period, date, and initials (you can just use your initials instead of your full name since the tube is small).

2. While you are waiting, put crushed ice in your cup.

3. Make sure the bead is at the bottom of the tube. Your instructor will add 24 µl of the *Vrs1* primer mix to your PCR tubes.

4. Add 1 µl of your DNA template to your PCR tube.

5. Vortex the tube until the bead fully dissolves and the solution is clear.

6. Tap the PCR tube on the table to get all of the solution down to the bottom of the tube.

7. Store tubes on ice until instructed to transfer your tubes to the **thermocycler**.

8. Give your tube of DNA back to the instructor for storage.

### *Restriction Digest of the* Vrs1 *Gene from 2-row and 6-row plants*

**Materials:**

| | |
|---|---|
| *Vrs1* PCR Product (DNA) | New England Biolabs (NEB) Buffer 4 |
| NciI Restriction Enzyme | Molecular Grade Water |
| Micropipettes | Tips |
| 0.2 ml PCR Tubes | 1.5 ml Centrifuge Tube |
| 37 °C Waterbath or thermocycler | |

### *Day 1: Restriction Digestion of PCR product*

1. Obtain a cup with crushed ice. Get your *Vrs1* PCR product tube from your instructor and place on ice.

2. Obtain a new, empty 0.2 ml PCR tube and label it like the *Vrs1* PCR tube (your OWB #, class period, date, and initials), but add the word Digest to the side.

3. Add 20 µl of the *Vrs1* PCR product to the new tube. Keep the remaining tube on ice.

4. Take the tube to your instructor to have 5 µl of the reaction mix added.

5. Store tubes on ice until instructed to transfer your tubes to be incubated at 37 °C for 1 hour.

### *Day 2: Vrs1 Restriction Product Electrophoresis:*

1. Obtain your digest product from the instructor.

2. On a piece of wax paper (parafilm), combine 3 µl of **loading dye** with 10 µl of digest product. Draw up and dispense the mixture with your pipette three to four times to mix it. Your product should be a blue color once mixed.

3. Take your wax paper over to the gel electrophoresis. Add 10 µl of the blue DNA/dye mixture to the correct gel well indicated below.

| Blank | DNA ladder | Cut 2-row | Uncut 2-row | Cut 6-row | Uncut-6-row |
|---|---|---|---|---|---|

4. Repeat Steps 1-3 for each sample you want to load onto the electrophoresis gel.

5. Be sure to use a new tip for each sample! Don't worry about getting the micropipette tip point down into the gel well. Hold the tip over the gel well you are targeting and dispense the DNA. The loading dye causes it to sink down into the well.

6. When all samples are loaded, run the gel electrophoresis at 70 volts for 1 hour 15 minutes. These conditions are optimal for the resolution of the DNA fragments; however, they can be adjusted to complete the run within a class period (e.g., 80 volts for 50 minutes).

7. Gently remove the gels and photograph for later analysis.

### *Day Three: Analyzing the data.*

Calculate the size of each fragment of the *Vrs1* (2-row) allele after restriction digestion (# of fragments and size of each)   Number: ________  Size of fragments (from largest to smallest) ______________

Calculate the size of fragment of the *vrs1* (6-row) allele after restriction digestion (# of fragments and size of each)   Number: ________  Size of fragments  (from largest to smallest) _______________

Researchers can estimate the size of fragments by comparing them to a lane (called the ladder) with fragments of known sizes.   Looking at the picture of your gel, what are the sizes of the restriction digestion fragments in the 2-row and 6-row samples.

| **Sample** | **2-row (*Vrs1/__*)** | **6-row (*vrs1/vrs1*)** |
|---|---|---|
| Calculated Size (based on DNA sequence) | | |
| Measured size of restriction fragments (gel) | | |

Does the data support your hypotheses?

Explain and give reasons why you may see unexpected banding in your samples.

## Experiment #3: Investigating alleles that influence disease resistance in OWB plants

### Overview

In this experiment, students observe several different OWB plants, sorting them into susceptible or resistant lines by their phenotype when exposed to the fungus that causes powdery mildew disease (**Figure 9**). They will then take samples and extract DNA from these plants and investigate a genetic locus, *Mla*, that is known to contribute to disease resistance. We will use two different sets of primers, one set corresponding to conserved sequences and one set that binds to variable regions of the *Mla6* allele.

### Learning Objectives

Upon Completion of this experiment, students will be able to:

- Describe 3 different plant disease epidemics and their impact on human society.
- Draw the interaction between primers and template DNA and explain the effect of changing template sequence on primer binding.
- Compare and contrast conserved and variable regions of a gene in terms of sequence and functional similarity.

### Introduction: Historic Impact of Plant diseases

#### *Irish Potato Famine - 1845*

In 1845, *Phytophthora infestans* made its way to Europe from the United States and Mexico. *P. infestans* is an oomycete (a fungus-like protist) that presents as moldy spots on potato leaves but eventually infects the actual potato tuber, causing it to rot and turn to mush. Potato fields in Belgium, France, and Ireland were devastated. Ireland was hit hardest due to its almost complete dependence on the crop. It is estimated that approximately one-million people died from starvation and related disease while another 1-2 million people emigrated to escape the famine. Even today, this pathogen is responsible for billions of dollars in damage to crops each year.

#### *Southern Corn Leaf Blight - 1970*

In 1970, an epidemic of *Cochliobolus heterostrophus* spread throughout the southern United States and the Corn Belt. The fungus causes dark lesions to appear on leaves and can cause the corn ear and cob to rot. Some areas of the Corn Belt saw 50% reductions in yield. Corn shortages increased the price of corn and even increased the price of alternate crops, when demand for livestock feed shifted from corn to other crops. Financial losses due to this epidemic are estimated at one billion dollars.

### *Modern Threats: Ug99 stem rust*

Ug99 is a lineage of *Puccinia graminis*, a fungus that causes wheat stem rust. Ug99 was discovered in 1998 in Uganda, and currently 7 races of the lineage have been identified. Ug99 showed novel virulence against the resistance gene Sr31. Because of this, it is estimated that 90% of wheat varieties are susceptible to Ug99. Ug99 has been active in Africa and the Middle East and its chances of spreading to Asia are likely. Wheat currently accounts for approximately 20% of humanity's food supply, and demand is expected to increase 60% by 2050. Thus, pathogens such as Ug99 poses a serious threat by reducing food availability worldwide.

### Introduction: Pathogen Resistance Genes in Barley

The lifecycle of cereal powdery mildews takes about 7 days and starts when spores germinate on the leaf surface and penetrate the host epidermal cells. Once it reaches the lumen (space between the cell wall and cytoplasm) a haustorium develops (this is a finger-like structure which allows the fungi to obtain nutrients from the host cells). After the haustorium develops additional hyphae form and spread across the leaf surface to invade neighboring cells. The powdery mildew can then either generate asexual spores or combine with a different mating type for sexual reproduction of diverse spores. Since both forms of reproduction generate new spores, this fungus can spread to other plants and start the infection cycle again.

### *OWB Resistant vs Susceptible Plants*

When barley plants are inoculated with powdery mildew, susceptible plants will begin to show signs of infection within 4-5 days. In the photos below, each plant was inoculated with powdery mildew and photographed seven days later. Note that some plants display green and healthy leaves, while other plants show leaves coated with the fungus (**Figure 9**).

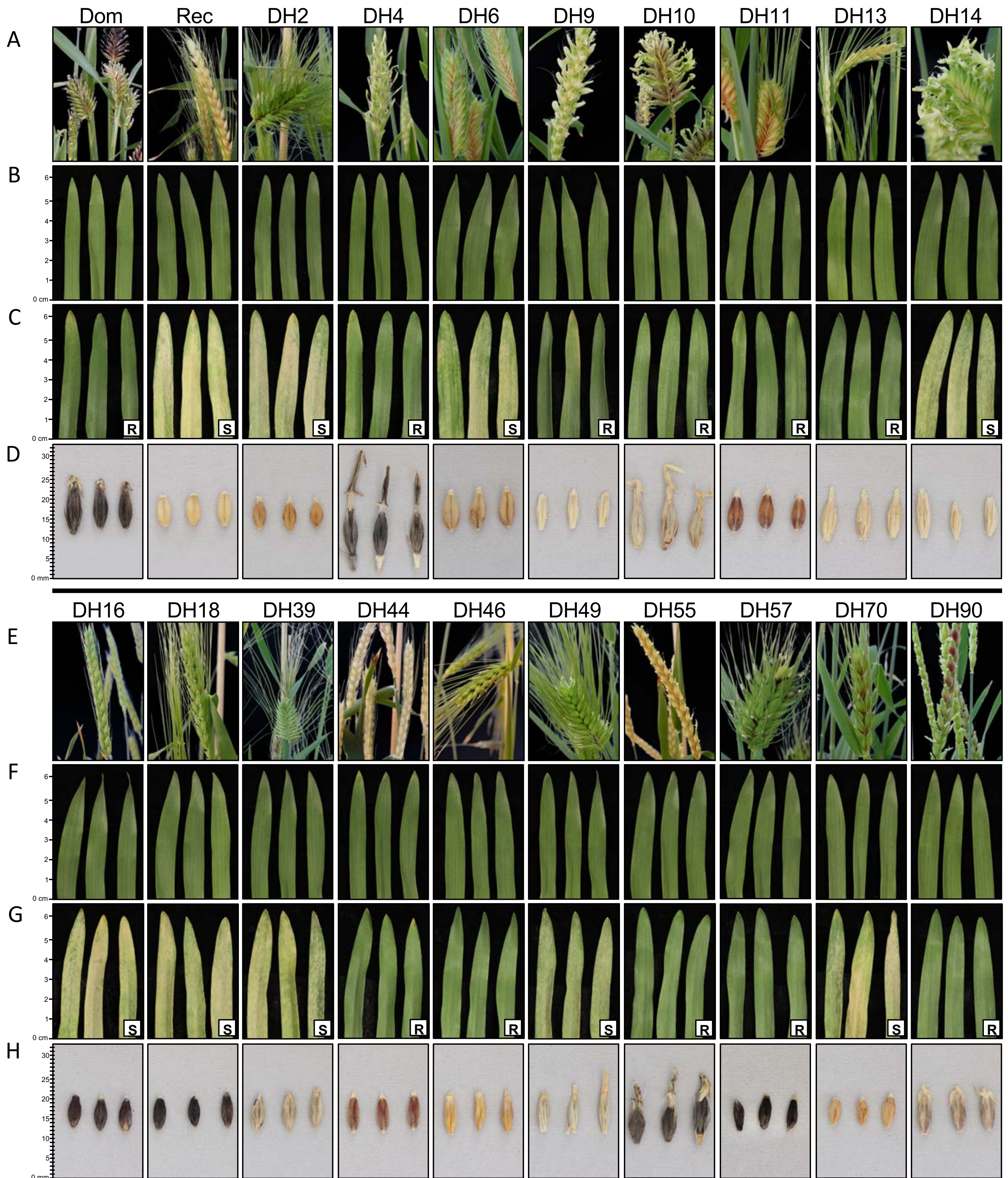



**Figure 9**. Phenotypes of the OWB Informative and Spectacular Subset (OWB-ISS). Barley spikes (A, E); seedling leaves (B, F); seedling leaves infected with powdery mildew (C, G); and seeds (D, H).

### *Regions of the Mla gene locus differ in similarity vs divergence*

Two sets of primers were designed for *Mla6,* an allele known to confer resistance to powdery mildew. The first set was designed in a conserved region, meaning that when the sequences of several different *Mla* alleles were aligned, the base pairs were identical between each allele at almost all locations. Conserved regions are more common at the 5 'end of DNA strands. The image below shows the aligned sequences of seven different *Mla* alleles. Note that there is only one instance of a base pair differing between any of the seven strands. Because the sequence is conserved in this region, when PCR is completed using primers from this region, the same bands are present for each plant because the same sequence is present at all alleles at this sequence.

```
mla1 gi|11612212|gb|  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 690
mla 8 gi|270267762|g  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 461
mla2 gi|270267758|gb  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGACCAATTGCTATTGA 461
mla10 gi|33943719|gb  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 874
mla6 gi|12957123|emb  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGACCAATTGCTATTGA 461
mla3 gi|270267760|gb  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 461
mla7 gi|33943717|gb|  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 880
```

**Figure 10A.** Conserved region of the *Mla* gene locus (DNA sequence of multiple *Mla* alleles lined up)

In contrast, the second set of primers was designed to target a divergent region of DNA. Divergent regions are more common towards the 3 'end. The image below shows a region of DNA in which there are dozens of differences in base pairs between the seven aligned alleles. Because there are significant differences between the sequences, when PCR is completed with divergent primers, only *Mla6* is amplified so only *Mla6* shows a band on the gel.

```
mla1 gi|11612212|gb|  CATCCCAGCCATCCCCGGATTTATATTCAGATGAGGCCGCATATAGCAAAAGGTGCTCATGATGACGATTT-GTGTGAGG 3089
mla 8 gi|270267762|g  CATCCCAACCATCCCCGGATTTATATTTACAAGACGCCGTGTATAGCAGAAGGTGCTCATGATGACGATTT-GTGTGTGG 2860
mla2 gi|270267758|gb  CATCCCAACCATCCCCAGATTGCAATTTTCATGCACCCGCCTATAGCAGAAGGTGCTCAAGATGACGATTTAATGTGA-- 2856
mla10 gi|33943719|gb  CATCCCAACCATCCCCAGATTGCAATTTTCATGCACCCGCCTATAGCAGAAGGTGCTCAAGATGACGATTTAATGTGATT 3271
mla6 gi|12957123|emb  CATCCCGACCATCTCCGGATTTATATTGACATGAGGCCGTGTATAGCAGAAGGTGCTCATGATGACGATTT-GTGTGAGG 2854
mla3 gi|270267760|gb  GACCCGCACGTATATTATATTAGCATTCAGATGAGGCCACGTATAGCAAAAGGTGCTCATGATGACGATTT-GTGTGAGG 2860
mla7 gi|33943717|gb|  CATCCCAGCCATCCCCCGATTTATATTGAGATGAGGCCGTGTATAGCAGAAGGTGCTCATGATGATGATTT-GTGTGAGG 3276
```

**Figure 10B.** Divergent (region of the *Mla* gene locus (DNA sequence of multiple *Mla* alleles lined up)

### PCR of *Mla6* resistance gene

To investigate differences between resistance and susceptibility, we will use two different sets of *Mla* primers to amplify different regions of the *Mla6* allele. This PCR amplification, along with the electrophoresis of the PCR products allows us to visualize the differences in DNA between barley plants that are resistant and those that are susceptible to powdery mildew.

**Materials:**

| | | |
|---|---|---|
| **Thermocycler** | 1.5 ml Centrifuge Tubes | Ice |
| Micropipettes | Pipette Tips | *Mla* PCR Primers |
| Molecular Grade Water | DNA Template(s) | |

PCR Tubes with Taq Beads Protocol: 0.2 ml PCR Tubes with Taq DNA Polymerase Beads

1. Obtain your DNA in the 2.0 ml microcentrifuge tube. Begin to thaw it out.

2. Obtain a PCR tube with a ***Taq* Polymerase** bead at the bottom and label it like the other tubes with your OWB #, class period, date, and initials (you can just use your initials instead of your full name since the tube is small). You should pick 2 OWB plants that were resistant to powdery mildew, and 2 that were resistant. You will amplify each sample using two different primer sets. You should have 5 tubes:

    1. Negative Control (no template)
    2. Conserved Primer (OWB -resistant)
    3. Divergent Primer (OWB -resistant)
    4. Conserved Primer (OWB -susceptible)
    5. Divergent Primer (OWB -susceptible)

3. While you are waiting, put crushed ice in your cup.

4. Make sure the bead is at the bottom of the tube. Your instructor will add 24 µl of the *Mla* primer mix to your PCR tubes.

5. Add 1 µl of your DNA template to your PCR tube.

6. Vortex the tube until the bead fully dissolves and the solution is clear.

7. Tap the PCR tube on the table to get all the solution down to the bottom of the tube.

8. Store tubes on ice until instructed to transfer your tubes to the **thermocycler**.

### Gel Electrophoresis: *Mla* PCR investigation

Using the same protocol from Experiment #1 (Using gel Electrophoresis to Analyze PCR products), prepare your PCR products (10 uL) with loading dye (3 uL) on a piece of wax paper, labeling each before adding to the wells in an agarose gel.

Gel Map

| Blank | DNA size ladder | Negative Control (no template) | Conserved Primer (OWB - resistant) | Divergent primer (OWB - resistant) | Conserved Primer (OWB - susceptible) | Divergent Primer (OWB - susceptible) |
|---|---|---|---|---|---|---|

When all samples are loaded, run the gel electrophoresis at 70 volts for 1 hour 15 minutes. These conditions are optimal for the resolution of the DNA fragments; however, they can be adjusted to complete the run within a class period (e.g., 80 volts for 50 minutes).

Discussion Questions (while the gel is running):

1) You are working with two sets of primers. One that binds to a region of the *Mla* gene that is very ***similar*** between different alleles (Conserved primers), while the other set binds to *Mla6* in a region that ***differs*** greatly among the other *Mla* alleles (Divergent primers).
    a. Why do you think some regions of a protein are the same for all the alleles, while other areas of the protein differ greatly?
    b. Which set of primers (conserved or divergent) do you think will let us differentiate between resistant and susceptible plants in this test? Explain.
2) Why do we include a sample for the PCR machine that does not include template DNA? Would you expect this sample to produce a detectible PCR product? Why or why not?
3) What could a plant do to resist infection? (Brainstorm ideas)

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# The iTAG Learning Module Instructor's Guide

## Core Questions Addressed

| **Core Question** | **Making Sense (Concepts Introduced)** | **Connections (to other concepts)** |
|---|---|---|
| What is an allele? | Genotype and phenotype<br>Central Dogma | Dominance and Recessiveness (Experiment 2) |
| What makes an allele recessive? | Mendelian Inheritance Patterns (Punnett Squares) | Molecular connections – variations in sequence at a given genetic locus (Experiment 2,3) |
| How do genes influence phenotype? | Central Dogma<br>Protein Functions | Enzymes<br>Homeotic genes (Experiment 1-3) |
| How do genes interact with one another? | Epistasis | *Lks1* & *Kap* (*lks/lks* exhibit recessive epistasis with *Kap/_* (Hooded)) (Experiment 1) |

**Next Generation Science Standards (NGSS) –**

While these exercises are designed for early-career undergraduate students, they can also be adapted for use in high school and address the following Next Generation Science Standards (NGSS) for high school**.**

**Science and Engineering Practices (SEP):** Asking Questions and Problem Solving, Analyzing and Interpreting data

**Life Science Core Ideas:** Variation of Traits

**Nature of Science**: Science Uses a Variety of Methods

## Instructor: Materials and Procedures (*Kap* Learning Module)

### Growing the Barley Plants

Barley needs 6-8 weeks to grow and mature to the point where its traits are easily identifiable to students (see *Growing Instructions for Oregon Wolfe Barley*). You can plant your "phenotype plants" two months prior to beginning the module and students determine if the genotype matches the phenotype observed, or you can plant them at the same time you plant your "genotype plants" so students can predict the phenotype according to their results of the genotype analysis. Instructors should plant the "genotype plant" about 8-10 days prior to the module. These week-old plants will provide the plant tissue that students use to extract DNA. as part of an ecology or plant anatomy unit. The more ownership the students have in the module, the more engaged they are in the investigation of genotype and phenotype.

## Timeframe for Module 1: Molecular Analysis of the *Kap* genetic Locus

*Vrs* and *Mla* PCR and Gel Electrophoresis timelines will be similar in length to the *Kap* primer timeline.  *Vrs* and *Mla* genes can be amplified with the previously extracted DNA. timeline.  *Vrs* and *Mla* genes can be amplified with the previously extracted DNA.

**Table 1. Time frame for running the module with *Kap* primers**

| Protocol | Time needed | Preparation & Materials |
|---|---|---|
| **Planting OWB Seeds** | 20 minutes | Have soil, seeds, markers, planting tags, and pots ready for student use. |
| **DNA extraction** | 3 x 45 minute class periods | Each lab station should contain 2mL tubes, markers, fresh slide and razor blade, pestle. Protocol ingredients should be easily accessible for each lab group. |
| **Kap PCR** | 15 minutes | Creating the primer mix ahead of time, will help speed up the process.  You may want to aliquot the primer mix for each group.  Time to discuss the PCR process. |
| **Gel electrophoresis** | 45 minutes | Gel can be made by students or prepared ahead of time.  Gel takes 30 minutes to run and can be run during or after class. |
| **Gel visualization and data analysis** | 20 minutes | Have viewing equipment set up and ready for the students. Time to discuss results. Two lines (16 & 44) display epistasis. |

| Table 2. Equipment and materials supplied by instructor | |
|---|---|
| **Item** | **When Needed:** |
| 20 - 5-inch pots | Growing plants |
| Seed tray with 20 cells or 20 small pots | |
| 20-40 plant tags | |
| Soil | |
| Masking Tape | |
| Fertilizer (20-20-20) | |
| 20 dowel rods or stakes | |
| Twist ties or plant wire | |
| Source of light | |
| Gloves | Throughout module |
| Goggles | |
| 10 fine point permanent markers | |
| Refrigerator / Freezer | |
| Ice (crushed is best) | |
| Microcentrifuge tube rack | |
| 10-20 glass microscope slides | DNA extraction |
| 10-20 razor blades | |
| 10-20 glass stirring rods | |
| 70% or 90% rubbing alcohol | |
| 10-20 Styrofoam or plastic cups | |
| Water bath | |
| Balance or scale (more than one is helpful) | DNA extraction & electrophoresis |

<table>
<tr><td>Distilled water</td><td rowspan="7">Electrophoresis</td></tr>
<tr><td>1 x 500 ml Erlenmeyer flask</td></tr>
<tr><td>1 x graduated cylinder (100ml)</td></tr>
<tr><td>Oven mitt or hot glove</td></tr>
<tr><td>Parafilm or wax paper</td></tr>
<tr><td>Lab tape (masking tape will work)</td></tr>
<tr><td>Microwave</td></tr>
<tr><td>Food coloring</td><td rowspan="3">Micropipette practice</td></tr>
<tr><td>Analytical balance</td></tr>
<tr><td>Weigh boats</td></tr>
</table>

## Core Equipment and materials (in Teacher Travel Kits)

| Table 3. Core equipment and materials |
| --- |
| Oregon Wolfe barley seeds (20 plants) |
| All tubes (PCR tubes, 1.5 ml tubes, 2.0 ml tubes) |
| All solutions |
| Micropipettes and tips (4 sizes) |
| Thermocycler |
| Vortex |
| Centrifuge |
| 2 - Electrophoresis chambers & combs |
| 1 - Electrophoresis power supply |
| Transilluminator |

| Table 4. iTAG supply list per 20 plant set | | | | |
|---|---|---|---|---|
| **Equipment** | ***Kap*** | ***Vrs1*** | **Practice** | **Total** |
| 2.0 ml microcentrifuge tubes | 40 | | | 40 |
| 1.5 ml microcentrifuge tubes | 121 | 2 | 40 | 163 |
| Small micropipette tips (10 µl) | 68 | 130 | 60 | 258 (3 boxes) |
| Medium micropipette tips (200 µl) | 20 | 44 | 20 | 84 (1 box) |
| Large micropipette tips (101-1000 µl) | 149 | 2 | 20 | 171 (2 boxes) |
| **Regular tube protocol:**<br>PCR tubes | 20 | 40 | | 60 |
| **PCR beads protocol:** GE Healthcare: Illustra™ PureTaq™ Ready-To-Go™ PCR Beads<br>Store @ room temp | 20 | 20 | | 40 |
| **PCR beads protocol:**<br>PCR tubes | | 20 | | 20 |
| **Reagents** | | | | |
| 2X CTAB buffer<br>Store @ room temp | 19.6 ml | | | 19.6 ml |
| 20% SDS<br>Store @ room temp | 2.1 ml | | | 2.1 ml |
| 5M potassium acetate<br>Store @ -20˚C | 8.3 ml | | | 8.3 ml |
| 70% ethanol<br>Store @ -20˚C | 10.1 ml | | | 10.1 ml |
| Absolute isopropanol<br>Store @ -20˚C | 11 ml | | | 11 ml |

| | | | | |
|---|---|---|---|---|
| TE buffer<br>Store @ room temp | 4.1 ml | | | 4.1 ml |
| 2-Mercaptoethanol<br>Store @ room temp | 405 µl | | | 405 µl |

## Basic Materials Checklists and Storage Instructions

## Growing Instructions for Oregon Wolfe Barley

**Containers:** In general, the larger the pot, the larger the plant. You will obtain a good grow-out with 13 cm (5-inch) pots. When the plants become larger, a dowel rod or bamboo plant stake and twist ties or other support will be needed to hold the stalks up right. For this set of experiments, instructors need at least 20 pots for plants to be grown to maturity and examined to determine phenotype, and one tray (with a minimum of 20 cells or seedling containers) for DNA extraction.

**Soil:** Use a peat moss mix (e.g., Sungrow LC1) that will drain well. Barley is less tolerant of acid than most plants, so if you have reason to believe your soil is acidic, have it tested and adjust the pH to 7.0 with lime.

**Seeding:** Prepare your containers with soil and sow 1-3 seeds per pot at a depth of approximately 2.5 centimeters (one inch). Lightly compact the soil over the seeds and water without causing the seed to float to the top. Seedlings should emerge within one week.

**Fertility:** If your soil mix does not already contain time-release fertilizer, fertilize with a dilute solution of liquid fertilizer, such as Rapid Grow or Peters (20-20-20). The plants should be fertilized once per week starting when the plant reaches two leaves of growth, and then fertilized twice per week when the plants start flowering. Continue at this rate until the plants start to dry down.

**Watering:** Barley is less tolerant of over-watering than under-watering. Treat your OWBs like houseplants, watering when the surface of the soil is moist but not dry to the touch. It is better to water infrequently but generously (until water flows through drain holes at the bottom of the pot) than to water lightly at frequent intervals.

**Propagation conditions:** Provide supplemental lighting for 16 hours per day. Fluorescent lights will work, but they should be numerous and no further than 1.5 m (5 feet) from the canopy surface. Sufficient light quantity and quality are essential.

**Culture:** The OWBs will show a stunning array of plant growth and development patterns. The first plants will head within 30 days of planting and the last will head at about 90 days. Plant height at heading can range from 40 to 120 centimeters (16 to 48 inches). Taller plants may require supplemental support. Use bamboo or dowel stakes and wire ties.

*Modified from https://barleyworld.org/

## Teacher Instructions: Module 1: Molecular Analysis of the *Kap* genetic Locus

DNA Extraction and Purification will take 3 days (45 minute class) or 2 (90 minute class)

*Day 1 Harvest Plant Tissue*

1. Before class, add 400 µl **2-Mercaptoethanol** to 19.6 ml of 2x CTAB = 20 ml total. This should be done in a fume hood.

2. Aliquot 805 µl of the **2-Mercaptoethanol** and **CTAB** solution into 20 x 1.5 ml microcentrifuge tubes labeled CTAB-2Mercap for students.

3. Aliquot 105 µl of **SDS** into 20 x 1.5 ml microcentrifuge tubes labeled SDS for students.

*Day Two: Extract DNA*

1. Before class, set up water bath at 65 ˚C.

2. Aliquot 415 µl of the **Potassium Acetate** into 20 - 1.5 ml microcentrifuge tubes labeled PA for students. This must be kept cold.

3. Aliquot 545 µl **Absolute Isopropanol** into 20 - 1.5 ml microcentrifuge tubes labeled AI for students. This must be kept cold.

4. Have styrofoam cups and crushed ice ready.

5. While incubating, students will collect the cup, ice, and potassium acetate tube and absolute isopropanol tube.

6. While centrifuging, have students label new 2.0 ml microcentrifuge tubes.

7. Pipetting the supernatant without getting any green plant material can be difficult. Be prepared to centrifuge tubes several times to help students get the 1 ml of supernant needed.

**End of Day Notes:**

Make sure each tube is properly labeled as the students give them to you. The permanent marker can often get rubbed off.

Store these samples overnight at 4 ˚C or freeze for longer storage.

*Day Three: Purification of DNA (This can be done on Day 2 if you have 90 minute periods)*
Before Class:

1. Aliquot 505 µl of **70 % ethanol** into 20 - 1.5 ml microcentrifuge tubes labeled E for students. This must be kept cold.

2. Aliquot 205 µl of **TE Buffer** into 20 - 1.5 ml microcentrifuge tubes labeled TE for students.

**End of Day Notes:**

Make sure each tube is properly labeled as the students give them to you. The permanent marker can often get rubbed off.

Store these samples overnight at 4 ˚C or freeze for longer storage.

Source: Protocol modified from Keb-Llanes et al. Plant Molecular Biology Reporter 20: 299a-299e. 2002.

## Polymerase Chain Reaction of the *Kap* Gene

Note: You may be using PCR Beads, or Taq DNA polymerase with Master Mix. Both of these will use 0.2 mL centrifuge tubes/

Regular 0.2 ml PCR Tubes (No beads) Protocol: **Taq DNA Polymerase**, & Master Mix

PCR Tubes with **Taq Beads Protocol**: 0.2 ml PCR Tubes with Taq DNA Polymerase Beads

### Using Regular *Taq* Polymerase PCR (Use with regular tubes)

1. Create primer mix in a 1.5 ml centrifuge tube by adding enough **Master Mix**, **water, *Kap* primers**, and ***Taq* polymerase** for all reactions (plus two to compensate for pipetting error). ***See chart below for determining primer mix amounts.***

2. Add the ***Taq* polymerase** to the primer mix just before students come to get the 24 µl. *Taq* must be kept cold to prevent degradation.

**Table 5. Preparing primer mix**

| Reagent | Volume (µl per reaction) | Number of reactions | Total volume for all reactions |
|---|---|---|---|
| **Molecular Grade H2O** | 10.5 µl | 22 | 231 µl |
| **Master Mix** | 12.5 µl | 22 | 275 µl |
| ***Kap* Primer F** | 0.5 µl | 22 | 11 µl |
| ***Kap* Primer R** | 0.5 µl | 22 | 11 µl |
| ***Taq* Polymerase** | 0.125 µl | 22 | 2.75 µl |

<u>**Teacher Preparation: PCR Beads**</u>

**9.** Create primer mix in a 1.5 ml centrifuge tube by adding enough water and Kap primers for all reactions (plus two to compensate for pipetting error). ***See chart below for determining primer mix amounts*.**

**Table 6. Preparing Kap Primers**

| Reagent | Volume (ul per reaction) | Number of reactions | Total volume for all reactions |
|---|---|---|---|
| **Molecular Grade H2O** | 23 µl | 22 | 506 µl |
| ***Kap* Primer F** | 0.5 µl | 22 | 11 µl |
| ***Kap* Primer R** | 0.5 µl | 22 | 11 µl |

Total Primer Mix = 528 µl

**Table 7. For Each PCR amplification reaction**

| Reagent | Volume (µl per reaction) |
|---|---|
| Primer Mix | 24 µl |
| Template DNA | 1.0 µl |

Total Reaction Volume = 25 µl

**Preparing Samples for PCR:**

Cycling Parameters

- Step 1: 94˚C for 3 minutes
- Step 2: 94˚C for 30 seconds, 54˚C for 30 seconds, 72˚C for 1 min 30 sec (35x)
- Step 3: 72˚C for 10 minutes
- Step 4: 4˚C for ∞ (hold forever)

The thermocycler program has a run time of over two hours. Take that into careful consideration when planning for multiple classes running the module.

**After Lab day:**

Make sure each tube is properly labeled as the students give them to you. The permanent marker can often get rubbed off.

Once the samples have run the program, store them at 4˚C with the DNA samples

**Primer Information**

| | | | |
|---|---|---|---|
| *Kap* = | 3BF | CCCCTCAAAGTTCAGGTCAATCCT | 24 bps |
| | 3DR | ATAAAACCAGAAGAGTGTGGAGTA | 24 bps |

Reference for *Kap* primers: Williams-Carrier, R., Lie, Y., Hake, S., and Lemaux, P. (1997). Ectopic expression of the maize *kn1* gene phenocopies the Hooded mutant of barley. Development. 124: 3737-3745.

Note: Primers can be ordered through Invitrogen or any other supplier of oligonucleotides. Primers are supplied to teachers working with the Wise Lab (USDA-ARS/Iowa State University).

PCR Beads: GE Healthcare illustra™ Pure*Taq*™ Ready-To-Go™ PCR Beads
Store @ Room Temperature

## Using gel Electrophoresis to Analyze PCR products

### Preparing the Gel:

1. First, determine the volume of the gel to be used. This will depend on the length and width of the gel tray, as well as the approximate depth of the gel you want. Multiply the gel volume by 0.01 to determine the number of grams of **agarose** needed for a 1% gel. Using this module, an 80 ml gel will require 0.8 grams of agarose.

   *The 1% gel can be somewhat flimsy. If you want to make a "stiffer" gel, make a 1.5- 2% gel. It will take longer to run Electrophoresis, but it is easier to move around between storage and viewing. Just double the amount of agarose used (2%) or add 1.5x (1.5%).*

2. In an Erlenmeyer flask, add your calculated amount of agarose to a volume of 1X TBE buffer equal to the desired gel volume. So, using the example from above of 1%, you would measure out 0.8 g of agarose and 80 ml of 1X TBE and pour them both in the flask. (You may get a stock solution of 10X TBE Buffer. In this case, you must dilute this 1X TBE. You can do this by adding 10ml of the 10X TBE to 90 ml of distilled water.)

3. Dissolve the agarose using a microwave oven. Use 45-60 second intervals, gently swirling in-between each interval, but be careful not to create bubbles, as this will interfere with pouring of the gel. When solution is clear, the agarose is dissolved.

4. Let the flask stand on the tabletop until it is warm (but not below 55˚C because the gel will start to solidify). A good indicator is if you can touch the bottom of the flask for several seconds without your hand getting too hot. While you are waiting for the solution to cool, tape the ends of the gel tray with labeling tape or masking tape.

5. Add 1 µl of Gel Green stain for every 10 ml of buffer used. Again, using our example, the 80 ml of 1X TBE we used in Step 2 would require 8 µl of Gel Green. The Gel Green will dissolve quickly simply by swirling the contents of the flask.

6. Make sure the 2 20-well gel comb(s) is (are) inserted into the gel box. Now pour the agarose solution into the gel tray. Let it stand until the solution completely cools and

becomes semi-solid. A good indicator that the gel is ready is if you notice it has become a whitish-cloudy color.

7. Remove the combs and tape from the gel tray before placing into the gel box. Pour 1X TBE into the gel box until both reservoirs are full and the gel is slightly submerged (about 1 mm over the top of the gel).

   * The TBE buffer in the chamber can be reused multiple times throughout this module.*

8. Prepare a class sheet that maps out the wells in the gel so that students can mark where they put their DNA sample. (a simple example is below)

| **Student Names** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **Ladder** | **16** | **18** | **39** | **44** | **46** | **49** | **55** | **57** | **70** | **90** |
| **Wells** | | | | | | | | | | | |

If you have time, you can run this during class and visualize the gel before the period ends. If you don't have enough time, you can run the gel, wrap it in plastic wrap or put it in a sealed plastic bag with a little bit of buffer to keep it moist and store it in the refrigerator. The cool temperature will prevent the DNA bands from diffusing throughout the gel becoming difficult to see.

*** It is important to note: DNA is negatively charged, so it will run from the negative end of the box (black electrode) to the positive end of the box (red electrode). The saying "Run to red" helps to remember.***

## Teacher Instructions: Exercise 2: Restriction Length Polymorphisms and the *Vrs1* gene

In this activity, the barley DNA samples are amplified by **Polymerase Chain Reaction** using *Vrs1* primers, then the PCR products are cut with *NciI* (a restriction endonuclease that cuts the dominant (2-row) allele in 3 places and the recessive (6-row) allele in two places. This PCR amplification, along with the electrophoresis of the PCR products allows us to see the DNA difference between barley plants that have a **two-row seed spike** and those that have a **six-row seed spike**.

Students will plant seeds and sample tissues from 2- seed row and 6-seed row plants following the same instructions for the *Kap* gene analysis (Hooded vs awed). They will purify the DNA using the same 3-day procedure they used for the *Kap* gene. Depending on time constraints, students can plant seeds, harvest tissues, and purify DNA for both experiments at the same time, taking care to clearly label each test-tube and sample, as working with multiple samples simultaneously requires clear and consistent documentation and care to not mix tubes when completing the bench work.

### Preparing samples for PCR of the *Vrs1* gene – for 22 reactions (11 students with both 2-row and 6-row spikes)

**Teacher Preparation: Regular *Taq* Polymerase PCR**

1. Create primer mix in a 1.5 ml centrifuge tube by adding enough **Master Mix**, **water, *Vrs1* primers**, and ***Taq* polymerase** for all reactions (plus two to compensate for pipetting error). ***See chart below for determining primer mix amounts*.**

| Reagent | Volume (µl per reaction) | Number of reactions | Total volume for all reactions |
|---|---|---|---|
| **Molecular Grade H2O** | 23 µl | 22 | 506 µl |
| ***Vrs1* Primer F** | 0.5 µl | 22 | 11 µl |
| ***Vrs1* Primer R** | 0.5 µl | 22 | 11 µl |

Add the ***Taq* polymerase** to the primer mix just before students come to get the 24 µl. *Taq* must be kept cold to prevent degradation.

### *Cycling Parameters*

Step 1: 94˚C for 3 minutes

Step 2: 94˚C for 30 seconds, **60˚C for 30 seconds**, 72˚C for 1 min 30 sec (35x)

Step 3: 72˚C for 10 minutes

Step 4: 4˚C for ∞ (hold forever)

The thermocycler program has a run time of over two hours. Take that into careful consideration when planning for multiple classes running the module. ***Note the annealing temperature is different than the *Kap* gene protocol.

Make sure each tube is properly labeled as the students give them to you. The permanent marker can often get rubbed off.

Once the samples have run the program (again, not the difference in annealing temperatures of the cycle parameters), store them at 4 ˚C with the DNA samples.

Students will run a gel electrophoresis of the *Vrs1* PCR product in the next section while the digest is occurring.

### *Primer Information*

| | | | |
|---|---|---|---|
| *Vrs1* = | HvHox1.01F | CCGATCACCTTCACATCTCC | 20 bps |
| | HvHox1.02R | GGTTTCTGCCGATCTTGAAGC | 21 bps |

Reference for *Vrs1* primers: Komatsuda, T., Pourkheirandish, M., He, C., Azhaguvel, P., Kanamori, H., Perovic, D., Stein, N., Graner, A., Wicker, T., Tagiri, A., Lundqvist, U., Fujimura, T., Matsuoka, M., Matsumoto, T., and Yano, M. (2007). Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc. Natl. Acad. Sci. USA. 104: 1424-1429.

PCR Beads: GE Healthcare illustra™ Pure*Taq*™ Ready-To-Go™ PCR Beads (Store @ Room Temperature)

## Restriction Digest of the *Vrs1* Gene from 2-row and 6-row plants

**<u>Teacher Preparation - *Vrs1* Digest:</u>**

1. Create a reaction mix of NEB Buffer 4, *Nci*I, and H20 in a 1.5 ml centrifuge tube (see table below). Keep on ice until ready to transfer 5 µl to each student's tube.

**<u>Preparing samples for Restriction Digestion</u>**

| Reagent | Volume (µl) / reaction | No. of reactions | Mix volume (µl) |
|---|---|---|---|
| **Molecular Grade H2O** | 2 | 22 | 44 |
| **NEB Buffer 4 (10x)** | 2.5 | 22 | 55 |
| ***NciI* (20 µg/µl)** | 0.5 | 22 | 11 |

| **Preparing samples for digest** | |
|---|---|
| **Reagent** | **Volume (µl per reaction)** |
| Reaction Mix | 5 µl |
| Template DNA (PCR Products) | 20 µl |

### Teacher Preparation: *Vrs1* Digest Product Gel Electrophoresis:

Make a gel using the same instructions from Gel Electrophoresis of Kap gene and fill the chamber with electrophoresis buffer before students add samples.

### Teacher Preparation: Experiment #3 Investigating alleles that influence disease resistance in OWB

There are two possible primer sets to use for this experiment: conserved region primers and divergent region primers. Using the conserved primers will yield a gel electrophoresis pattern with identical bands for each plant. Using the divergent primers will show different bands between the resistant and susceptible plants.

If the experiment is run with both primer sets, students can compare the results and use their observations to inform discussions about gene structure. If this is beyond the scope of the instructor's course goals, the experiment should be run with just the divergent primers because these are the primers that will distinguish resistant (R) and susceptible (S) plants.

### *Teacher Preparation: Regular Taq Polymerase PCR*

1. Create primer mix in a 1.5 ml centrifuge tube by adding enough **Master Mix**, **water, *Mla* primers**, and ***Taq* polymerase** for all reactions (plus two to compensate for pipetting error). ***See chart below for determining primer mix amounts*.**

Add the ***Taq* polymerase** to the primer mix just before students come to get the 24 µl. *Taq* must be kept cold to prevent degradation.

**Table 11. Preparing primer mix**

| Reagent | Volume (µl) | Number of reactions | Total volume (µl) for all reactions |
|---|---|---|---|
| **Molecular grade H2O** | 10.5 µl | 22 | 231 µl |
| **Master mix** | 12.5 µl | 22 | 275 µl |
| ***Mla* primer F** | 0.5 µl | 22 | 11 µl |
| ***Mla*** primer **R** | 0.5 µl | 22 | 11 µl |
| ***Taq* polymerase** | 0.125 µl | 22 | 2.75 µl |

### *Teacher preparation: PCR Beads*

1. Create primer mix in a 1.5 ml centrifuge tube by adding enough water and *Mla* primers for all reactions (plus two to compensate for pipetting error).

**Table 12. Preparing the reaction mix**

| Reagent | Volume (µl) | Number of reactions | Total volume (µl) for all reactions |
|---|---|---|---|
| **Molecular grade H2O** | 23 µl | 22 | 506 µl |
| ***Mla* primer F** | 0.5 µl | 22 | 11 µl |
| ***Mla* primer R** | 0.5 µl | 22 | 11 µl |

Table 13. Preparing samples for PCR

| Reagent | Volume (µl per reaction) |
|---|---|
| Primer mix | 24 µl |
| Template DNA | 1.0 µl |

Make sure each tube is properly labeled as the students give them to you. The permanent marker can often get rubbed off. Once the samples have run the program, store them at 4˚C with the DNA samples.

**Cycling Parameters**
Step 1: 94˚C for 3 minutes
Step 2: 94˚C for 30 seconds, 59˚C for 30 seconds, 72˚C for 1 min 30 sec (35x)
Step 3: 72˚C for 10 minutes
Step 4: 4˚C for ∞ (hold forever)

The thermocycler program has a run time of over two hours. Take that into careful consideration when planning for multiple classes running the module.

**Primer Information**

Divergent Forward: 5 ’AAGGAATTGCCGTCCACAGT 3’

Divergent Reverse: 5 ’CACTGGCAGGACTAAGTCGG 3’

Conserved Forward: 5 ’AGAATCAGTTGTGATCAGTCTGGGCG 3’

Conserved Reverse: 5 ’CCGGAGATGGTCGGGATGAG 3’

PCR Beads: GE Healthcare illustra™ Pure*Taq*™ Ready-To-Go™ PCR Beads

Store @ Room temperature

### *Mla6 gene regions*

Two sets of primers were designed for *Mla6*. The first set was designed in a conserved region, meaning that when the sequences of several different *Mla* alleles were aligned, the base pairs were identical between each allele at almost all locations. Conserved regions are more common at the 5 ’end of DNA strands. The image below shows the aligned sequences of seven different *Mla* alleles. Note that there is only one instance of a base pair differing between any of the seven strands. Because the sequence is conserved in this region, when PCR is completed using primers from this region, the same bands are present for each plant because the same sequence is present at all alleles at this sequence.

```
mla1 gi|11612212|gb|  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 690
mla 8 gi|270267762|g  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 461
mla2 gi|270267758|gb  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGACCAATTGCTATTGA 461
mla10 gi|33943719|gb  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 874
mla6 gi|12957123|emb  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGACCAATTGCTATTGA 461
mla3 gi|270267760|gb  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 461
mla7 gi|33943717|gb|  GAGCAACTCCAAAAGGTGGCTGATAGGCGTGACAGGAACAAGGTATTTGTTCCTCATCCTACGAGAACAATTGCTATTGA 880
```

In contrast, the second set of primers was designed to target a divergent region of DNA. Divergent regions are more common towards the 3 ’end. The image below shows a region of DNA in which there are dozens of differences in base pairs between the seven aligned alleles.

Because there are significant differences between the sequences, when PCR is completed with divergent primers, only *Mla6* is amplified and only *Mla6* shows a band on the gel.

```
mla1 gi|11612212|gb|  CATCCCAGCCATCCCCGGATTTATATTCAGATGAGGCCGCATATAGCAAAAGGTGCTCATGATGACGATTT-GTGTGAGG 3089
mla 8 gi|270267762|g  CATCCCAACCATCCCCGGATTTATATTTACAAGACGCCGTGTATAGCAGAAGGTGCTCATGATGACGATTT-GTGTGTGG 2860
mla2 gi|270267758|gb  CATCCCAACCATCCCCAGATTGCAATTTTCATGCACCCGCCTATAGCAGAAGGTGCTCAAGATGACGATTTAATGTGA-- 2856
mla10 gi|33943719|gb  CATCCCAACCATCCCCAGATTGCAATTTTCATGCACCCGCCTATAGCAGAAGGTGCTCAAGATGACGATTTAATGTGATT 3271
mla6 gi|12957123|emb  CATCCCGACCATCTCCGGATTTATATTGACATGAGGCCGTGTATAGCAGAAGGTGCTCATGATGACGATTT-GTGTGAGG 2854
mla3 gi|270267760|gb  GACCCGCACGTATATTATATTAGCATTCAGATGAGGCCACGTATAGCAAAAGGTGCTCATGATGACGATTT-GTGTGAGG 2860
mla7 gi|33943717|gb|  CATCCCAGCCATCCCCCGATTTATATTGAGATGAGGCCGTGTATAGCAGAAGGTGCTCATGATGATGATTT-GTGTGAGG 3276
```

## Glossary

**2-Mercaptoethanol:** Used in plant DNA extraction. Is a strong reducing agent and removes polyphenols like tannin from interacting with the DNA.

**2X CTAB Buffer:** A detergent that breaks up and dissolves the lipid membranes of the cells. Chemically alters the proteins and polysaccharides so that they don't interact with DNA.

**5M potassium acetate:** Removes proteins from the DNA. Also used as a salt for the ethanol precipitation of DNA.

**20% (w/v) sodium dodecyl sulfate:** Aids in lysing of the cells for DNA extraction.

**70% ethanol:** Removes salts and other water-soluble impurities from the DNA pellet.

**Absolute isopropanol:** Causes the DNA to precipitate out of solution.

**Agarose:** Used to make the gel. Separates DNA fragments.

**Alkaline soil conditions:** Soil with a higher pH.

**Amplification:** Increases the number of DNA fragments into millions of copies.

**Cosegregation:** Transmission of two or more linked genes on a chromosome to the same daughter cell, leading to the inheritance of these genes together.

**Digital pipettes:** Adjustable pipettes that can measure small volumes.

**DNA polymorphisms:** Differences in DNA sequences.

**dNTP:** Stands for deoxynucleotide triphosphates. They are single units of DNA composed of a sugar, phosphate group and one of the bases A, T, C, will form the new DNA strands during PCR.

**Doubled haploid:** Cells that contains two identical homologous chromosomes (one chromosome from one parent that has been doubled).

**Epistasis:** When the expression of one gene depends on the presence of one or more genes.

**F1:** The first generation produced by a cross between parents that are homozygous for the trait. The F1 generation will be heterozygous (one dominant gene, one recessive gene).

**Gel Green DNA stain:** Stain for detecting double-stranded DNA in agarose gels. Less hazardous alternative to ethidium bromide.

**Gel Loading Dye:** Helps to weigh down the DNA solution in the gel wells. It also helps to monitor the progress of the DNA as it moves through the gel.

**Genotype:** The genetic makeup of an organism.

**Homoeotic mutation:** Mutation in a gene that causes the development of specific structures.

**Hooded:** Phenotype resulting from *Kap* (from German "kapuze" meaning hood). Creates a morphological hood by placing extra palea on the distal end of the lemma followed by rudimentary florets with inverse polarities.

**Introns:** Non-coding sequence of RNA removed from a transcript before translating to a protein.

***Kap* gene:** Gene that codes for the hooded phenotype.

***Lks2* epistasis of the *Kap* gene:** In plants homozygous for the recessive allele at *lks2*, the expression of hooded phenotype is masked. This results in the expression of a short, rather than hooded, phenotype.

**Master Mix:** Contains salts, magnesium, dNTPs, and optimized reaction buffer, all ingredients to perform a PCR. The magnesium is needed for the enzyme polymerase to function properly. The salt and buffer are needed for appropriate pH. dNTPs are single unit nucleotides that will be the "building blocks" for new DNA strands.

**Molecular Grade Water:** Certified to be contamination-free.

**Phenotype:** The observed properties or outward appearance of a trait. The physical expression of the genes possessed by an organism.

**Polymerase chain reaction (PCR):** Method of amplifying or copying DNA fragments. Begins with a trace template (genomic DNA in this module) and produces exponentially large amounts of a specific piece of DNA.

**Polymorphic:** When two or more clearly different phenotypes exist in the same species population.

**DNA polymorphisms:** variation in DNA sequences among individuals, groups, or populations. This could be a single base pair change, many base pairs, insertions or deletions, or repeated sequences.

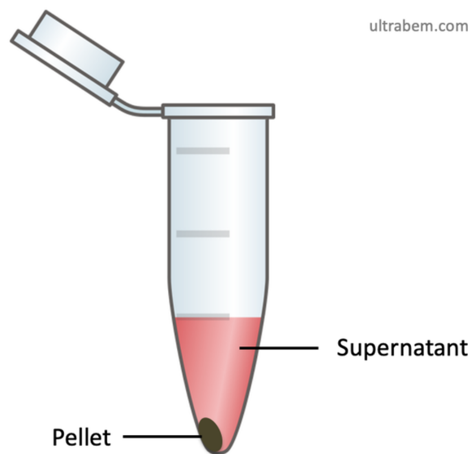
**Primers:** Used to determine the DNA fragment to be amplified by PCR. Serves as starting points for DNA synthesis. They are short pieces of single-stranded DNA that are complementary to the target sequence.

**Restriction enzyme digest:** Enzymes isolated from bacteria that recognize specific sequences and DNA.

**Sickle Cell:** Hereditary blood disorder, characterized by red blood cells that are a rigid, sickle shape. This decreases the cells' flexibility and can cause complications.

**Size fractionate:** The separation of DNA fragments by size.

**Supernatant**: Liquid lying above a solid after precipitation and centrifugation. (image Wikimedia commons)




**Taq DNA Polymerase:** A DNA polymerase that can withstand the high temperatures required to synthesize a new DNA strand from a template.

**Tay Sachs:** A recessive genetic disorder that causes progressive deterioration of nerves. It begins around six months of age and usually results in death at an early age.

**TBE Buffer:** Commonly used in electrophoresis. Provides ions to carry the current and maintains a relatively constant pH. Made of Tris, Boric Acid, and EDTA.

**TE Buffer:** Commonly used buffer solution that makes DNA or RNA soluble, while protecting it from degradation.

**Thermocycler:** Machine that rapidly heats and cools for PCR reactions. The **thermal cycler** (also known as a **thermocycler**, **PCR machine** or **DNA amplifier**) is a laboratory apparatus most commonly used to amplify segments of DNA via the polymerase chain reaction (PCR).

**Vortex:** A machine that agitates a solution vigorously.

***Vrs1* gene:** Gene that causes either a two-rowed spike (dominant allele) or a six-rowed (recessive allele).