iTAG Barley:
A Grade 7-12 Curriculum to Explore Inheritance of Traits and Genes using Oregon Wolfe Barley Student Edition
(v6 updated 4/26/16)
Authors

McGhee, L\textsuperscript{1,8}, N Hayes\textsuperscript{2,8}, R Schuck\textsuperscript{3,8}, L Maffin\textsuperscript{4,8}, G Hall\textsuperscript{5,8}, T Hubbard\textsuperscript{6,8}, E Whigham\textsuperscript{7,8}, G Fuerst\textsuperscript{9}, and Roger P Wise\textsuperscript{9,10}

\textsuperscript{1}Colfax-Mingo Community High School, 204 N League Rd, Colfax, IA 50054
\textsuperscript{2}Cedar Rapids Kennedy High School, 4545 Wenig Rd NE, Cedar Rapids, IA 52402
\textsuperscript{3}Ames Community High School, 1925 Ames High Dr, Ames, IA 50010
\textsuperscript{4}Bondurant-Farrar Community High School, 1000 Grant Street N, Bondurant, IA 50035
\textsuperscript{5}Southeast Polk Community High School, 7945 NE University Ave, Pleasant Hill, IA 50327
\textsuperscript{6}Ankeny Community High School, 1155 SW Cherry St, Ankeny, IA 50023
\textsuperscript{7}Iowa Western Community College, 2700 College Rd, Council Bluffs, IA 51503
\textsuperscript{8}Research Experience for Teachers, Iowa State University, Ames, IA 50011
http://www.eeob.iastate.edu/plantgenomeoutreach/

\textsuperscript{9}Corn Insects and Crop Genetics Research Unit, U.S. Department of Agriculture-Agricultural Research Service, Iowa State University, Ames, Iowa 50011–1020
\textsuperscript{10}Department of Plant Pathology and Microbiology, Center for Plant Responses to Environmental Stresses, Iowa State University, Ames, Iowa 50011–4009

For more information on materials and additional slides of OWB phenotypes:
E-mail roger.wise@ars.usda.gov ; fax:515-294-9420.

Funding provided by joint NSF-PGRP/ERA-CAPS project: Grant# 1339348.
Introduction

One of the basic concepts in biology is that an organism’s physical traits are controlled by its DNA. In other words, one’s genotype for a particular trait controls the phenotype that is expressed. Yet, this connection between DNA and physical characteristic is not always made. The ‘Inheritance of Traits and Genes in Barley’ (iTAG Barley) Project is a module of laboratory and classroom activities designed to help you make this connection.

The laboratory portion begins with you planting and growing barley plants so that phenotypic variation can be observed first hand. One trait in particular, the difference between “awned” and “hooded” plants, is the focus of the basic Learning Module. The barley plants in the photos below exhibit these two phenotypes of the dominant “hooded” trait and the recessive “awned” trait. The Learning Module also includes protocols for DNA Extraction, Polymerase Chain Reaction, and Gel Electrophoresis. You get the opportunity to experience these basic biotechnology techniques, and the final results of the electrophoresis allows you to see the DNA polymorphisms among plants with different phenotypes.

“Awned” Recessive  “Hooded” Dominant

In addition, several extension activities are provided in the Extension Module. These protocols can be used in addition to the Learning Module, or separately in whatever way that helps.
Why Oregon Wolfe Barley?

The Oregon Wolfe Barleys (OWBs) are a model resource for genetics research and instruction. The population of 94 double haploid lines was developed from an F1 of a cross between dominant and recessive marker stocks advanced by Dr. Robert Wolfe. Segregating plants from the OWB doubled haploid (DH) population are easily grown on a lighted window bench in the classroom. These lines originate from a wide cross and have exceptionally diverse and dramatic phenotypes.

You can observe the spikes for seed-coat color, two row vs. six row (encoded by Vrs, a domestication trait where two row is dominant and six row is recessive), hooded vs. non-hooded (Kap: encoded by BKn3 of the Knox gene family - a homoeotic mutation where the awn is replaced by another floret), and long awn vs. short awn traits (encoded by Lks2). In addition, plants homozygous for the recessive allele at lks2, the expression of the hooded phenotype is masked, resulting in the expression of a short-awned, rather than hooded, phenotype.

You will gain experience in phenotype observation and first hand knowledge of genetic history related to cellular pathways, grain domestication, and developmental mutations in plants. You will perform the polymerase chain reaction (PCR) to amplify the Kap and Vrs1 (HvHox1) genes using DNAs you isolate from the segregating plants, size fractionate the products on agarose gels, and document your results. Interactive exercises are presented on cosegregation of PCR products and whole plant phenotypes in the OWB population.

This module grew out of conversations between high school science teachers and USDA-ARS researchers at Iowa State University. During the summer NSF-sponsored, Research Experience for Teachers program (RET), discussions on how to incorporate research into the classroom were common. Everyone agreed that high school students were capable of understanding and conducting PCR; the challenges were how to fund and implement the concept. We decided on the OWB population barley because it is easy to grow, the plants are phenotypically diverse and easy to score, and the DNA extraction is straightforward. This module was included as a “broader impacts” component of NSF Grant #0922746. It is now continuing under a joint NSF/ERA -CAPS project: Host Targets of Fungal Effectors as Keys to Durable Disease Resistance. Grant# 1339348. As of spring 2014, this module has been used successfully in 40 Iowa high school biology classrooms, impacting >1,000 students. The project is continuing to expand its reach with the first iTAG Barley Workshop in July, 2015 hosted by Iowa State University. The workshop organizers and participants collectively will use the iTAG Barley Module in 53 classes during the 2015-16 school year impacting more than 1,400 high school and middle school students. Additional workshops for the 2016 summer are already being planned at Iowa State University in Ames, IA and Tuskegee University in Tuskegee, AL. We hope you have as much fun with these incredible plants as we have!
Goals:

After completing the iTAG BARLEY module you 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.

Organization:

This module is sequential in that each activity, in most cases, must be completed before the next activity can be started. Two exceptions are the Strawberry DNA extraction and Tip Top Electrophoresis, which are included to help understand concepts before attempting more technical procedures.

You begin by planting a population of Oregon Wolfe Barley. If you have little or no prior experience using digital pipettes, the Pipette Technique and Practice activity can be used to introduce you to this tool. In addition, this will help ensure that the pipettes are in good working order.

The leaf tissue DNA Extraction is simple in theory but complex in practice. Therefore, Strawberry DNA Extraction introduces you to concepts before exposing you to more challenging techniques. Similarly, Tip Top Electrophoresis develops the schema necessary for understanding genomic electrophoresis. Tip Top also visualizes movement of bands of molecules through a gel.

Because one of the goals of this module is for you to understand the relationship between an individual's genotype and phenotype, amplification of a single gene is done via PCR. The genotype of each plant in the population can be compared to the phenotype to observe cosegregation. The primers utilized to amplify the Kap gene take advantage of different size introns, thus, polymorphic products are produced to distinguish dominant or recessive genes by electrophoresis. Gel Green DNA stain is used, along with the Vernier Transilluminator, to visualize bands of DNA. The Gel Green is both non-toxic and light insensitive, making it safe and convenient to use.
**Modifications:**

You may also use PCR to amplify the *Vrs1* gene, perform a *restriction enzyme digest*, and use electrophoresis to distinguish genotypes. The concept of *epistasis* can be discussed in light of *Lks2 epistasis of the Kap gene* in a few individuals.

**Extensions:**

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.

Agriculture is important, but human health may be more important to high school students. The same principles used to associate the *Kap* gene with the hooded phenotype are used regularly to associate genes with human genetic diseases such as *Sickle Cell* or *Tay Sachs*. These extensions make this module cross curricular since topics in history, social studies, psychology, sociology, and food science are influenced by genetics and segregation of traits.

If you are interested in conducting the module contact the developers at gsfuerst@iastate.edu or rpwise@iastate.edu

Want to learn more about double haploid production? Check out this YouTube video

http://www.youtube.com/watch?v=V2jOEuZjirg

Learn.Genetics through the University of Utah has great virtual labs available that explain the PCR process as well as Gel Electrophoresis.

http://learn.genetics.utah.edu/content/labs/
CHAPTER 2

The Module
2.2 Growing Instructions for Oregon Wolfe Barley

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 dirt.

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. Seedlings should emerge within one week.

**Fertility:** 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 [www.barleyworld.org](http://www.barleyworld.org)*
2.3 Leaf Tissue DNA Extraction

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

**Reagents and Buffers:**
- 2X CTAB Buffer
- 20% (w/v) sodium dodecyl sulfate (SDS)
- 5M potassium acetate (stored at -20°C)
- Absolute isopropanol (stored at -20°C)
- 70% ethanol (stored at -20°C)
- TE Buffer
- 2-Mercaptoethanol**
- Rubbing Alcohol
*Always wear gloves and safety glasses.*

**Day One:**

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.

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 and 100 µl of **SDS** from the tube labeled SDS. **Vortex** 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 to double check your tube has the proper labeling before giving to instructor. The marker can rub off.***
Day Two:

1. Allow your sample to thaw out. You can speed this up by holding the microcentrifuge tube in your hand. Use the vortex machine 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 (labeled PA) 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 from the tube labeled PA. 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).
Day Two Continued:

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 have to re-centrifuge the tube.**

8. Add 540 µl of ice cold *absolute isopropanol* from the tube labeled AI. 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). **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.

***Make sure to double check your tube has the proper labeling before giving to instructor. The marker can rub off.***
**Day Three:**

1. Allow your sample to thaw out. 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. Gently 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 from the tube labeled TE. Use the vortex well to re-suspend the DNA pellet in the TE. Give the tube to your instructor for overnight storage.

***Make sure to double check your tube has the proper labeling before giving to instructor. The marker can rub off.***

2.4 Polymerase Chain Reaction of the *Kap* Gene

**Materials:**

- **Thermocycler**
- 1.5 ml Centrifuge Tubes
- Ice
- Micropipettes
- Pipette Tips
- *Kap* PCR Primers
- DNA Template(s)
- **Molecular Grade Water**
- PCR Tube with Bead: GE Healthcare illustra™ PureTag™ Ready-To-Go™ PCR Beads
Procedure:

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 in 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.

2.5 Pouring and Running an Electrophoresis Gel

**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
Your instructor will prepare the Electrophoresis Gel, but below is a video showing how it is done.

**Movie 2.5 Preparing an Electrophoresis Gel**

---

**Student Instructions - Preparing & Loading the DNA:**

Before the DNA can be loaded into the gel, a loading dye must be added. The loading dye molecules will run ahead of the DNA during electrophoresis and give you a visual indication of when to shut off the electric current.

1. Obtain you PCR product from the instructor.

2. On a piece of wax paper (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.

**Movie 2.6 Loading and Running a Gel**

---
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.

*** 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.***

**Visualizing the Gel:**

1. The DNA is not visible at this point, but during the electrophoresis the Gel Green stain has adhered to the DNA. In order to see the DNA, gently remove the gel from the gel box and place it upon the blue platform of the Vernier Transilluminator (see below). Lower the orange lid and turn the light knob. The DNA bands should become visible. The darker the surroundings, the better the bands show up. Turning off the room lights can help.
3.1 Extensions to the Module

This section includes several optional extension activities.

The first three activities should be conducted before the Learning Module to provide a foundation in basic techniques. Pipette Practices (3.2) are useful activities if you do not have experience using these important tools. The Strawberry DNA Extraction (3.3) exercise is a fun and low-tech way to introduce the process of DNA extraction. Tip Top Electrophoresis (3.4) is a “homemade” electrophoresis exercise. Using everyday materials, you can set up your own electrophoresis equipment. This activity is a great hands-on way to help you understand how electrophoresis works.

PCR of the Vrs1 Gene (3.5) and Restriction Digest of Vrs1 (3.6) provide options to go beyond the Learning Module. The Vrs1 gene has two alleles, one of which codes for a two-row seed spike in the adult barley plant, and the other which codes for a six-row seed spike. The two-row phenotype is dominant over the six-row. The two alleles, however, are the same length in terms of DNA base pairs. In order to determine the genotype of a plant, the DNA must first be amplified using PCR and then digested with a restriction enzyme. The enzyme used in this activity, NciI, cleaves the dominant two-row allele in three places, resulting in four DNA bands during electrophoresis. NciI cuts the recessive six-row allele in two places; thus, only three fragments are produced. Instructions for both the restriction enzyme digest and the subsequent electrophoresis of the resulting DNA fragments are included. Along with the Learning Module activities, these two exercises are a great way to help you make the connection between an organism’s expressed phenotype and the DNA that makes it happen.
3.2 Pipette Technique and Practice

The micropipette is a basic tool for transferring small volumes. These two different exercises are provided to help familiarize you with its proper use.

**Materials Practice #1:**
- Micropipettes
- Deionized Water
- Weigh Boat
- Analytical Balance

**Materials Practice #2:**
- Micropipettes
- Water
- Food Coloring
- Microcentrifuge Tubes
- Wax Paper/Parafilm
Micropipette Practice #1:

How to Adjust and Read the Micropipette

It is important for you to be able to adjust and read the volume setting on a micropipette. Brands may vary, but every pipette has some kind of knob to adjust volume and a window to read the volume. To adjust the micropipette to the volume you want, simply turn the knob until the correct reading shows up in the window. The trick is reading the window correctly. How you make a reading depends on which size of pipette you are using. For example, if you are using a 0.5-10 µl micropipette, the top number in the window represents the tens digit; the middle number represents the ones digit; and the bottom number is the tenths place. So if the reading looks like this,

```
    0
   5
  6
```

The micropipette is set to take up and dispense 5.6 µl.

A 2-20 µl micropipette is the same. The top number is the tens place; the middle is the ones place; and the bottom number is the tenths. If your reading looks like this,

```
  1
  3
  4
```

your micropipette is set to transfer 13.4 µl.
Larger pipettes are a bit different. For a 20-200 µl pipette, the top number is the hundreds digit; the middle number is the tens digit; and the bottom number is the ones digit. If you need to transfer 125 µl, you should turn the knob to make the reading look like so:

![Image of a 20-200 µl pipette setting to 125 µl]

For a 100-1000 µl micropipette, the top number is the thousands digit; the middle number is the hundreds, and the bottom number is the tens. If your protocol calls for 500 µl, you should set the micropipette to look like this:

![Image of a 100-1000 µl micropipette setting to 500 µl]

Seem strange? The best way to become familiar with each of the pipettes is to practice setting volumes. You’ll get the hang of it quickly. One final note, although a pipette can be dialed above or below it’s stated range; doing so will throw off the calibration and result in inaccuracy. NEVER DIAL A PIPETTE ABOVE OR BELOW IT’S RANGE.
How to Transfer a Sample

The plunger of the micropipette has two “stops”. As you press down, you reach the first stop. If you apply a little more pressure, the plunger continues to the second stop. It is important for you to recognize both stops in order to transfer volumes.

This is how to take up a sample with your micropipette:

1. Put a fresh tip on the end of your micropipette.
2. Press the plunger down to the first stop.
3. Insert the tip into the solution to be transferred.
4. Gently allow the plunger to lift back to its original position. This draws solution up into the tip.
To dispense your volume:

1. Place the tip into the container to which you are transferring the solution.

2. Smoothly press the plunger all the way down to the second stop. This will eject the solution from the tip.

3. Draw your tip out of the container before releasing the plunger back to its original position. If you release the plunger too soon, you may take up solution into the tip that you don’t want.

4. Use the ejector button to get rid of the tip.

Procedure – Pipette Practice

1. Adjust the dial on your pipette to the highest volume.

2. Pipette deionized water into a weigh boat on an analytical balance. Weigh and record the volume. Repeat five times.

3. Adjust the dial to the lowest volume. Repeat Step 2.

4. Adjust the dial to a middle range. Repeat Step 2.

5. One microliter of water should weigh one microgram (1µl = 1 µg). If your pipette is delivering incorrect volumes consistently it should be calibrated. If the volume is randomly inconsistent technique should be improved.

Modified from http://www.ehow.com/how_2044881_calibrate-pipette.html

For more information, check out: http://www.benchfly.com/video/151/how-to-use-a-pipetman/

For general information on various lab techniques see the parent web site: http://www.benchfly.com/
Micropipette Practice #2:

1 Liter (L) = 1,000 milliliters (ml) = 1,000,000 microliters (μl)

Precaution:
* Do not drop micropipette.  
* Do not run digital display below the minimum or above the maximum.  
* ALWAYS use with a tip.

1. Have each group member do the following
   a. Push the plunger to feel the first stop, then release … this stop is used to take up the sample.
   b. Push the plunger all the way down and release … this stop is used to expel the sample.
   **Be sure to feel the difference between the two stops.**

White Plunger 0.5-10 μl
2. Set the digital display to the following settings and check with your partner.
   1.00 μl  
   5.36 μl
   
   ![Diagram]

Yellow Plunger 2-20 μl
3. Set the digital display to the following settings and check with your partner.
   6.92 μl  
   15.50 μl
   
   ![Diagram]

Yellow Plunger 20-200 μl
4. Set the digital display to the following settings and check with your partner.
   34.8 μl  
   96.0 μl  
   182.4 μl
   
   ![Diagram]
Blue Plunger 100-1000 μl

5. Set the digital display to the following settings and check with your partner.
   250 μl  624 μl  898 μl

For the following, make sure to use the pipettes that will get the most accurate results.

6. You need a volume of 54.5 μl. What pipettes would you use to reach this volume?

7. You need a volume of 645.8 μl. What pipettes would you use to reach this volume?

8. You need a volume of 1,820.65 μl. What pipettes would you use to reach this volume?

**ATTACH A PIPETTE TIP NOW**
(Hold pipette with display facing you … then press the end of the pipette firmly into one of the tips in the box … gently lift it out)

9. Transfer the above amounts of water from the water tube to an empty tube.

10. Using the colored water, practice pipetting and mixing with the pipette. Make sure to practice mixing small amounts of colored water on the wax paper.

11. Bring your wax paper with color mixtures over to the electrophoresis box. You will practice loading a gel.
3.3 Strawberry DNA Extraction

**Materials:**

- 50 ml Centrifuge Tubes
- Zip-lock Sandwich Bags
- 95% Ethanol (ice cold)
- Styrofoam or Plastic Cups (8 oz.)
- Bleached Coffee Filters (white)
- Whole Strawberry

**Lysis buffer:**

- Detergent (we use Dawn dish soap)
- Salt (iodized or non-iodized will work)
- Distilled Water

For each 100 ml of lysis buffer, add ¼ teaspoon of salt to 90 ml of distilled water. Stir the buffer until the salt is dissolved. Add 10 ml of detergent and stir until mixed.
**Procedure:**

1. Take off the leaves on the top of the fruit and place the fruit in a sandwich bag.

2. Seal the sandwich bag and pulverize the fruit. Smash the fruit by hand and then roll a pen or marker back and forth over the bag to make the fruit as liquid as possible.

3. Add 10 ml of the lysis buffer to the bag and reseal.

4. Continue to roll the fruit tissue in the lysis buffer for two minutes.

5. Place a coffee filter in an 8 oz cup.

6. Pour the contents of the sandwich bag into the filter and set aside for 10 minutes.

7. Discard the coffee filter and its contents.

8. Pour 30 ml of ice cold 95% ethanol into a 50 ml centrifuge tube.

9. Pour the contents of the 8 oz cup into the tube, cap the tube, wait for the DNA to start precipitating in the ethanol (the process begins almost immediately and the DNA will continue to condense for the next few minutes).

*Protocol by Julie Townsend, Parkview Middle School, Ankeny, Iowa.*
3.4 Tip Top Electrophoresis

**Objective:** Simulate the process of DNA fingerprinting by using electric current to separate colored dyes.

**Materials:**

**For the electrophoresis chamber:**

Small plastic box approximately 8x12cm (empty micropipette tip boxes are perfect)

Two 5” pieces of stainless steel wire (11” stainless steel wire ties can be found at Lowes)

2 regular popsicle sticks

2 narrow popsicle sticks (coffee stirrer kind)

2 electrical leads with alligator clips

Five 9V batteries

Scissors

Masking tape

**For the gel and buffer:**

Agar-agar powder (available from Asian grocery stores) or agarose (available from chemical supply companies)

Water  Baking soda  Mat knife or razor blade
**For the samples:**

Water

Food coloring

Glycerin (available from pharmacies)

Needle-tip disposable pipette or micropipetter and tips

(Optional) beaker of water for rinsing tips between samples

**Assembly**

1. Make a comb to create wells in the gel that will eventually hold the samples. Cut the narrow popsicle sticks so that they sit just above the bottom of the base when hung from a regular popsicle stick (~3-4 cm depending on the depth of your box). Cut 5 teeth and tape them to a regular popsicle stick so that they are evenly spaced and hang down to the same level. Tape the other regular popsicle stick on the other side to secure the teeth, and check to see that they hang evenly when placed on the box without touching the bottom. Place the comb vertically in the top of your box. (Have your students come up with their own design giving them certain parameters. A wood comb does not pull out of the gel very well.)
2. Make a 0.2% sodium bicarbonate buffer by dissolving 2 g of baking soda in 1 L of water. You will need ~100 ml per set up. Just enough buffer to cover the gel and fill in the wells.

3. Make a 1% gel solution by adding 1 g of agar-agar powder to 100 ml of sodium bicarbonate buffer. You will need 40-50 ml of gel solution per set up. To dissolve the powder, heat the solution in the microwave, stopping every so often to swirl the solution. Watch the solution carefully as it will quickly boil over when too hot. When you see bubbles, stop the microwave, and swirl the solution until the agar-agar particles completely dissolve. The solution should be translucent when heating should stop.

4. Once the solution is cool enough to pour, add just enough into the box so that ~0.5 cm of the comb teeth are submerged. Poor the gel when it is warm to the touch. Adjust the comb by sliding it so that it is ~1.5 cm from the top of the box. Thinner gels will yield better separations.

5. The actual gel only needs to be half the size of the box. In addition, you need to make space to place the electrodes. Once the gel sets (~5-10 min), use a knife or blade of some kind to cut off the bottom half of the gel. Also, without disturbing the comb, cut out a thin strip from the top of the gel to make room for a wire electrode. Your gel should now be around 6 cm long and 8 cm wide (still the full width of your box). The extra pieces of gel can be recycled by reheating them in the microwave.

6. Bend each piece of stainless steel wire to run along the width of the box and hook over the side. Place one on either side of the gel. Use tape to secure them to the box if you need to. These will be the positive and negative electrodes.

7. Make a high voltage power supply by connecting the five 9V batteries. Clip two batteries together by inserting the positive terminal of one into the negative terminal of another. Attach the remaining batteries one by one in this way until you have a five-battery pack. Clip an electrical lead to each of the exposed terminals of the pack. You should now be able to use the battery pack to power the gel box by attaching the other ends of the electrical leads.
8. Prepare 5 different samples by mixing 1-2 drops of food coloring with 1 ml glycerin and 1 ml water in a small tube. We used blue, red, green, yellow, and purple (made by mixing blue and red food coloring).

**Procedure**

1. When your gel set-up is ready, pour just enough buffer to cover the solidified gel. Make sure you fill up the space left from the cut gels and that the gel is completely submerged.

2. Gently remove the comb by pulling straight up without tearing the gel. The wells should fill with buffer.

3. Use the needle tip pipette to transfer ~10 µl of each sample to an empty well. The volume of the thin tip of the pipette is about 10 µl. Submerge the tip in the buffer directly above the well and gently squeeze the sample into the well. It should fall into the well since it is denser than the surrounding buffer. You should use a new pipette for each sample to prevent contamination between samples. If you only have a few pipettes, rinse out the tip well in a large beaker of water before re-using.

4. Once all the samples are loaded, connect the leads from the power supply to the stainless steel wire electrodes attached to the box. Connect the negative terminal to the electrode at the top of the gel (near the combs) and the positive terminal to the electrode at the bottom of the gel. You should see bubbles forming along the electrodes when a complete circuit is made.

5. Allow the samples to run for 15-20 minutes and observe what happens to each sample.
Additional Information:

Molecular Cell Biology by Lodish et. al., W. H. Freeman (2000)

A classic molecular biology text available FREE online at:


http://en.wikipedia.org/wiki/Gel_electrophoresis

http://en.wikipedia.org/wiki/Food_dye

Gel Electrophoresis - Draft Julie Yu, Exploratorium, 2007
3.5 Polymerase Chain Reaction of the *Vrs1* Gene

In this activity, the barley DNA samples are amplified by **Polymerase Chain Reaction** using *Vrs1* primers. This PCR amplification, along with the electrophoresis of the PCR amplicons performed, allows you to visualize the DNA difference between barley plants that have a **two-row seed spike** and those that have a **six-row seed spike**.

Working with the **Vrs1 gene** is a bit more complex than working with **Kap gene**. The dominant and recessive alleles of the *Kap* gene have length polymorphisms, meaning they are coded by DNA sequences of different lengths. You will be able to distinguish the allele of each sample by your electrophoresis results. However, the alleles of the *Vrs1* gene are the same length. In order to distinguish the genotype of the *Vrs1* samples, the amplified DNA must be digested with a **restriction enzyme** to uncover the restriction enzyme length polymorphisms (RFLPs), before electrophoresis is done.
**Materials:**

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

PCR Tube with Bead: GE Healthcare illustra™ PureTaq™ Ready-To-Go™ PCR Beads

**Procedure:**

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.

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

\[ Vrs1 = \text{HvHox1.01F} \quad \text{CCGATCACCTTCACATCTCC} \quad 20 \text{ bps} \]

\[ \text{HvHox1.02R} \quad \text{GGTTTCTGCGATCTTGAAGC} \quad 21 \text{ bps} \]

3.6 Restriction Digest of the Vrs1 Amplicon

**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
- Thermocycler or 37°C Incubator or Water Bath
Day One:

Procedure:

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 of NEB Buffer 4 and NciI added.

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

Predicted Fragment Lengths:
Day One Continued:

Vrs1 PCR Product Electrophoresis:

1. On a piece of wax paper (parafilm), combine 1.5 µl of **loading dye** with 5 µl of Vrs1 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.

2. Take your wax paper over to the gel electrophoresis. Add 6.5 µl of the blue DNA/dye mixture to the correct gel well indicated by the paper and your instructor.

3. Repeat Steps 1-2 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.

4. 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.
**Day Two:**

**Vrs1 Digest Product Gel 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 by the paper and your instructor.

4. Repeat Steps 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.

While the digest gel is running, visualize the Vrs1 PCR product gel.

**Day Three:**

Visualize the gel of the Vrs1 Digest.
### 4.1 Stock Solutions

<table>
<thead>
<tr>
<th><strong>2X CTAB Buffer:</strong></th>
<th>Per 500 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-HCl</td>
<td>100 mM</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.4 M</td>
</tr>
<tr>
<td>EDTA (Ethylenediamine-tetraacetic acid)</td>
<td>20 mM</td>
</tr>
<tr>
<td>CTAB (Hexadecyltriethyl-ammonium bromide)</td>
<td>10 g</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>400 µl</td>
</tr>
<tr>
<td>(added the day of extraction)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>1X TE Buffer:</strong></th>
<th>Per 100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM Tris-HCl (pH 8.0) (Use 1M stock)</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>1 mM EDTA (Use 0.5M pH 8.0 stock)</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>10X TBE Buffer:</strong></th>
<th>Per 1000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.89 M Tris</td>
<td>108 g</td>
</tr>
<tr>
<td>0.89 M Boric Acid</td>
<td>55 g</td>
</tr>
<tr>
<td>0.02 M EDTA</td>
<td>9.3 g</td>
</tr>
</tbody>
</table>

Deionized Water Dilute 1:9 to prepare 1X TBE Buffer
Acknowledgements

Protocols

1 – Growing Instructions for Oregon Wolfe Barley

http://barleyworld.org/oregonwolfe

Modified from protocol at www.barleyworld.org. Seed can be obtained from http://barleyworld.org/oregonwolfe/plant-material.

2 – Leaf Tissue DNA Extraction

Protocol developed by Wise Lab, USDA-ARS/Iowa State University, Ames, IA

3 – Polymerase Chain Reaction of Kap Gene

Primer sequence provided by Patrick Hayes, Oregon State University

Protocol developed by Wise Lab, USDA-ARS/Iowa State University, Ames, IA


4 – Pouring and Running an Electrophoresis Gel

Modified from Gel Green Protocol 2011

5 – Pipette Calibration and Use

Practice #1:

Modified from: http://www.ehow.com/how_2044881_calibrate-pipette.html

For more information see http://www.benchfly.com/

Practice #2:

Protocol by Laurie McGhee, Colfax-Mingo High School, Colfax, IA
6 – Strawberry DNA Extraction

Protocol by Julie Townsend, Parkview Middle School, Ankeny, Iowa.

7 – Tip Top Electrophoresis


8 – Polymerase Chain Reaction of Vrs1 gene

Protocol developed by Wise Lab, USDA-ARS/Iowa State University, Ames, IA


9 – Restriction Digest of Vrs1 Amplicon

Protocol developed by Wise Lab, USDA-ARS/Iowa State University, Ames, IA

10 – Fun videos:

http://www.youtube.com/watch?NR=1&v=khBmRuFc_P4

http://www.youtube.com/watch?v=IpMOrX1fzGM&feature=related

11 – Special Thanks to:

The authors thank Greg Fuerst, USDA-ARS Lab Technician, Iowa State University, Ames, IA; Eric Hall, Hoover High School, Des Moines, IA; Craig Walter, Ames High School, Ames, IA; Dr. Karri Haen, Research Institute for Studies in Education, Iowa State University, Ames, IA; and Dr. Adah Leshem, Plant Genomics Education Outreach, Iowa State University, Ames, IA, for critical reading of the manual.
2-Mercaptoethanol

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

Related Glossary Terms
Drag related terms here

Index

Chapter 2 - Leaf Tissue DNA Extraction
Chapter 2 - Leaf Tissue DNA Extraction
Chapter 2 - Leaf Tissue DNA Extraction
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.

Related Glossary Terms
Drag related terms here
5M potassium acetate

Removes proteins from the DNA. Also used as a salt for the ethanol precipitation of DNA.

Related Glossary Terms
Drag related terms here
**20% (w/v) sodium dodecyl sulfate**

Aids in lysing of the cells for DNA extraction.

---

**Related Glossary Terms**

Drag related terms here
70% ethanol

Removes salts and other water soluble impurities from the DNA pellet.

Related Glossary Terms

Drag related terms here
Absolute isopropanol

Causes the DNA to precipitate out of solution.

Related Glossary Terms

Drag related terms here
Agarose

Used to make the gel. Separates DNA fragments.

Related Glossary Terms
Drag related terms here
Alkaline soil conditions

Soil with a higher pH.

Related Glossary Terms

Drag related terms here
Amplification

Increases the number of DNA fragments into millions of copies.

Related Glossary Terms

Drag related terms here
Cosegregation

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

Related Glossary Terms

Drag related terms here
Digital pipettes

Adjustable pipettes that can measure small volumes. They have disposable tips.

Related Glossary Terms

Drag related terms here
DNA polymorphisms

Differences in DNA sequences.

Related Glossary Terms
Polymorphic
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, or G. They will form the new DNA strands during PCR.

Related Glossary Terms

Drag related terms here
Double haploid

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

Related Glossary Terms
Drag related terms here
**Epistasis**

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

**Related Glossary Terms**

Drag related terms here
**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 and one recessive gene).

**Related Glossary Terms**

Drag related terms here
Gel Green DNA stain

Stain for detecting double-stranded DNA in agarose gels. Less hazardous alternative to ethidium bromide.

Related Glossary Terms

Drag related terms here
Gel Loading Dye

Helps to weigh down the DNA solution in the gel wells. It also helps to visualize the progress of the DNA as it moves through the gel.

Related Glossary Terms

Drag related terms here
Genotype

The genetic makeup of an organism.

Related Glossary Terms

Drag related terms here
Homoeotic mutation

Mutation in a gene that causes the development of specific structures.

Related Glossary Terms

Drag related terms here
Hooded

Related Glossary Terms
Drag related terms here
**Introns**

Non-coding sequence of RNA removed a transcript before translation into protein.

---

**Related Glossary Terms**

Drag related terms here
Kap gene

Gene that codes for the hooded phenotype.

Related Glossary Terms
Drag related terms here
**Lks2 epistasis of the Kap gene**

In plants homozygous for the recessive allele at *lks2*, the expression of the hooded phenotype is masked. This results in the expression of a short-awned phenotype rather than hooded phenotype.

---

**Related Glossary Terms**

Drag related terms here
Long awn

Related Glossary Terms
Drag related terms here
Master Mix

Contains salts, magnesium, dNTPs, and optimized reaction buffer, all key ingredients to perform a PCR. The magnesium is needed for the enzyme (Taq 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.

Related Glossary Terms
Drag related terms here
Molecular Grade Water

Certified to be contamination-free.

Related Glossary Terms
Drag related terms here
Phenotype

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

Related Glossary Terms

Drag related terms here
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.

Related Glossary Terms

Drag related terms here
**Polymorphic**

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

**Related Glossary Terms**

DNA polymorphisms
Primers

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

Related Glossary Terms

Drag related terms here
Restriction enzyme digest

Enzymes isolated from bacteria that recognize specific sequences and then cut DNA.

Related Glossary Terms

Drag related terms here
Seed-coat color
Short awn

![Image of Short awn]

**Related Glossary Terms**

Drag related terms here

---

**Index**  
[Find Term]

**Chapter 1 - Introduction**
Sickle Cell

Hereditary blood disorder, characterized by red blood cells that are an abnormal, rigid, sickle shape. This decreases the cells’ flexibility and can cause various complications.

Related Glossary Terms
Drag related terms here
Six row

Recessive Allele

Related Glossary Terms
Drag related terms here
Size fractionate

The separation of DNA fragments by size.

Related Glossary Terms

Drag related terms here
Supernatant

Liquid lying above a solid.

Related Glossary Terms

Drag related terms here
Taq DNA Polymerase

A DNA polymerase that can withstand the high temperatures required is used to synthesize a new DNA strand from a template.

Related Glossary Terms

Drag related terms here
Tay Sachs

A recessive genetic disorder that causes progressive deterioration of nervous system cells that begins around six months of age and usually results in death by age four.

Related Glossary Terms

Drag related terms here
**TE Buffer**

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

---

**Related Glossary Terms**

Drag related terms here
Thermocycler

Machine that rapidly heats and cools for PCR reactions.

Related Glossary Terms

Drag related terms here
Two row

Dominant Allele

Related Glossary Terms
Drag related terms here
Vortex

A machine that agitates a solution vigorously.

Related Glossary Terms
Drag related terms here

Index
Find Term

Chapter 2 - Leaf Tissue DNA Extraction
Chapter 2 - Leaf Tissue DNA Extraction
Chapter 2 - Leaf Tissue DNA Extraction
**Vrs1 gene**

Gene that causes either a two-rowed spike (dominate) or a six-rowed (recessive).

---

**Related Glossary Terms**

Drag related terms here