

# iTAG Barley:

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





# Authors

McGhee, L<sup>1,8</sup>, N Hayes<sup>2,8</sup>, R Schuck<sup>3,8</sup>, L Maffin<sup>4,8</sup>, G Hall<sup>5,8</sup>, T Hubbard<sup>6,8</sup>, E Whigham<sup>7,8</sup>, G Fuerst<sup>9</sup>, and Roger P Wise<sup>9,10</sup>

---

<sup>1</sup>Colfax-Mingo Community High School, 204 N League Rd, Colfax, IA 50054

<sup>2</sup>Cedar Rapids Kennedy High School, 4545 Wenig Rd NE, Cedar Rapids, IA 52402

<sup>3</sup>Ames Community High School, 1925 Ames High Dr, Ames, IA 50010

<sup>4</sup>Bondurant-Farrar Community High School, 1000 Grant Street N, Bondurant, IA 50035

<sup>5</sup>Southeast Polk Community High School, 7945 NE University Ave, Pleasant Hill, IA 50327

<sup>6</sup>Ankeny Community High School, 1155 SW Cherry St, Ankeny, IA 50023

<sup>7</sup>Iowa Western Community College, 2700 College Rd, Council Bluffs, IA 51503

<sup>8</sup>Research Experience for Teachers, Iowa State University, Ames, IA 50011

<http://www.eeob.iastate.edu/plantgenomeoutreach/>

<sup>9</sup>Corn Insects and Crop Genetics Research Unit, U.S. Department of Agriculture-Agricultural Research Service, Iowa State University, Ames, Iowa 50011-1020

<sup>10</sup>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](mailto:roger.wise@ars.usda.gov) ; fax:515-294-9420.

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



# CHAPTER 1

## Overview

***iTAG Barley***

[resistancegenomics.org](http://resistancegenomics.org)





# 1.1 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 by students. The 'Inheritance of Traits and Genes in Barley' (iTAG Barley) Project is a module of laboratory and classroom activities designed to help students make this connection.

Students plant and grow 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. Students get the opportunity to experience these basic biotechnology techniques, and the final results of the electrophoresis allow students 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 teachers to meet their curriculum. Some of the activities may be useful if you are working with younger or more inexperienced students, while others move beyond the Learning Module and work best with more advanced students.

## 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, making the population attractive for teaching basic plant development, genetics, and genomics in high school biology.

Students 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.

Thus, students gain experience in phenotype observation and first hand knowledge of genetic history related to cellular pathways, grain domestication, and developmental mutations in plants. Students perform the **polymerase chain reaction (PCR)** to amplify the *Kap* and *Vrs1* (*HvHox1*) genes using DNAs they isolate from the segregating plants, **size fractionate** the products on agarose gels, and document their 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 and your students have as much fun with these incredible plants as we have!

## 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.

## 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 students understand concepts before attempting more technical procedures.



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. If students have little or no prior experience using **digital pipettes**, the Pipette Technique and Practice activities can be used to introduce them to this tool.

The leaf tissue DNA Extraction is simple in theory but complex in practice. Therefore, Strawberry DNA Extraction introduces students to concepts before exposing them 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. This is helpful later when attempting to convince students that the bands they see are actually the DNA they amplified.

Because one of the goals of this module is for students 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:**

This module is designed for use in K-12 and undergraduate classrooms. Alignment to the Iowa Core Curriculum and Next Generation Science Standards can be found in Appendix C. In elementary or middle school classrooms the students might only plant barley to observe, or extract DNA from strawberries, or watch Tip Top Electrophoresis to understand the role of DNA. In a high school classroom, students might grow barley, extract its DNA, amplify the *Kap* gene using PCR, and use the product to perform electrophoresis.

Advanced high school or college classrooms may also use PCR to amplify the ***Vrs1* gene**, perform a **restriction enzyme digest**, and use electrophoresis to distinguish genotypes. In college classrooms 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](mailto:gsfuerst@iastate.edu) or [rpwise@iastate.edu](mailto:rpwise@iastate.edu)

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

<http://www.youtube.com/watch?v=V2jOEUZjjrg>

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/>

Want a hands-on activity that introduces students to centrifuging by using common substances? Check out this Google Document by Ron Schuck that you can edit to fit your needs.

<https://docs.google.com/document/d/1728zDRvDM4TxplEGdKFNAIsxWnnWvvpkeVLRrbi2XTo/pub>



# CHAPTER 2

## The Module





## 2.1 The Learning Module Overview & Materials

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 both a traditional (50 minute) class period, or in block scheduling (90 minute). 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).

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” before, during, or after completing the module. You can plant two months prior to beginning the module and students determine if the genotype matches the phenotype observed, or you can plant at the same time or just after the module so that students can predict the phenotype according to their results of the genotype. A planting should be done about 8-10 days prior to the module. These week-old plants will provide the “harvest tissue” for extracting DNA. You will need at least 20 pots for plants to be grown to maturity and phenotyped, and one tray (with a minimum of 20 cells or seedling containers) to plant your OWBs for DNA extraction.

The two plantings could be used as part of an ecology or plant anatomy unit. Based on our experiences, the more ownership the students have in the module, the better it runs.

### How do I print these materials?

If you are using iBooks and would like to print any of these pages download the PDF at either of the following sites:

Wise Lab Outreach

<http://www.public.iastate.edu/~imagefpc/Subpages/outreach.html>

American Society of Plant Biologists K-12 Resources

[http://my.aspb.org/members/group\\_content\\_view.asp?group=80400&id=99873](http://my.aspb.org/members/group_content_view.asp?group=80400&id=99873)



## Time Frame for Running the Module

Protocol	Time Needed	Preparations
<b>Planting OWB</b>	20 minutes	Have soil, seeds, markers, planting tags, and pots ready for student use.
<b>DNA Extraction</b>	3 - 45 minutes 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.
<b><i>Kap</i> PCR</b>	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.
<b>Gel Electrophoresis</b>	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.
<b>Viewing of Gel</b>	20 minutes	Have viewing equipment set up and ready for the students. Time to discuss results. Two lines (16 & 44) display epistasis.



Equipment & Materials Supplied by Teacher	
Item	Section
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
Distilled water	Electrophoresis
1 - 500 ml Erlenmeyer flask	
1 - graduated cylinder (100ml)	
Oven mitt or hot glove	
Parafilm or wax paper	
Lab tape (masking tape will work)	
Microwave	
Food coloring	Micropipette Practice
Analytical balance	
Weigh boats	



<b>Equipment &amp; Materials Supplied by Wise Lab</b>
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



iTAG Supply List Per 20 Plant Set				
	<i>Kap</i>	<i>Vrs1</i>	Practice	Total Needed
2.0 ml Microcentrifuge Tubes	40			40
1.5 ml Microcentrifuge Tubes	121	2	40	163
Small Micropipette Tips (10 $\mu$ l)	68	130	60	258 (3 Boxes)
Medium Micropipette Tips (200 $\mu$ l)	20	44	20	84 (1 Box)
Large Micropipette Tips (101-1000 $\mu$ l)	149	2	20	171 (2 Boxes)
<b>Regular Tube Protocol:</b> PCR Tubes	20	40		60
<b>PCR Beads Protocol:</b> GE Healthcare: illustra™ PureTaq™ Ready-To-Go™ PCR Beads Store @ Room Temp	20	20		40
<b>PCR Beads Protocol:</b> PCR Tubes		20		20
<b>Reagents</b>				
2X CTAB Buffer Store @ Room Temp	19.6 ml			19.6 ml
20% SDS Store @ Room Temp	2.1 ml			2.1 ml
5M Potassium Acetate Store @ -20°C	8.3 ml			8.3 ml
70% Ethanol Store @ -20°C	10.1 ml			10.1 ml
Absolute Isopropanol Store @ -20°C	11 ml			11 ml
TE Buffer Store @ Room Temp	4.1 ml			4.1 ml
2-Mercaptoethanol Store @ Room Temp	405 $\mu$ l			405 $\mu$ l



**iTAG Supply List  
Per 20 Plant Set**

	<i>Kap</i>	<i>Vrs1</i>	Practice	Total Needed
<i>Kap</i> Primer F Store @ -20°C	12 µl			12 µl
<i>Kap</i> Primer R Store @ -20°C	12 µl			12 µl
<i>Vrs1</i> Primer F Store @ -20°C		12 µl		12 µl
<i>Vrs1</i> Primer R Store @ -20°C		12 µl		12 µl
1X TBE Store @ Room Temp	1 L (100 ml of 10X TBE)	1 L (100 ml of 10X TBE)	1 L (100 ml of 10X TBE)	3 L (300 ml of 10X TBE)
Agarose Store @ Room Temp	0.8 g	1.6 g	0.8 g	3.2 g
Gel Green Stain Store @ Room Temp	9 µl	18 µl		27 µl
Loading Dye Store @ Room Temp	70 µl	140 µl		210 µl
NEB Buffer 4 Store @ -20°C		57 µl		57 µl
Ncil (enzyme) Store @ -20°C		12 µl		12 µl
1 KB Ladder Store @ -20°C	12 µl	24 µl		36 µl
<b>Regular PCR Tubes:</b>				
<i>Taq</i> (enzyme) Store @ -20°C	3 µl	3 µl		6 µl
Master Mix Store @ -20°C	280 µl	280 µl		560 µl
Molecular Grade H2O Store @ Room Temp	240 µl	240 µl 47 µl		527 µl
<b>PCR Bead Tubes:</b>				
Molecular Grade H2O Store @ Room Temp	506 µl	506 µl		1.1 ml



## 2.2 Growing Instructions for Oregon Wolfe Barley



**2 x 4 frame with adjustable light rack (a bench by a window will work as well)**

**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 and twist ties or other support maybe needed to hold the stalks up right.

**Soil:** Use a standard potting mix. Barley is less tolerant of acid than **alkaline soil conditions**, 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 generously without causing the seed to 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)

An example of a chart where students record the weekly growth of their barley plants is included in Chapter 4 Appendix, Section 6.



## **Student Instructions:**

### **Materials:**

5 inch Pot

Seed Packet

Fine Point Permanent Marker

Masking Tape

Plant Tag

Standard Potting Mix

OWB # 2  
Date  
3rd  
Jane &  
John

### **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.



## 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 -20C)

**Absolute isopropanol** (stored at -20C)

**70% ethanol** (stored at -20C)

**TE Buffer**

**2-Mercaptoethanol\*\***

Rubbing Alcohol

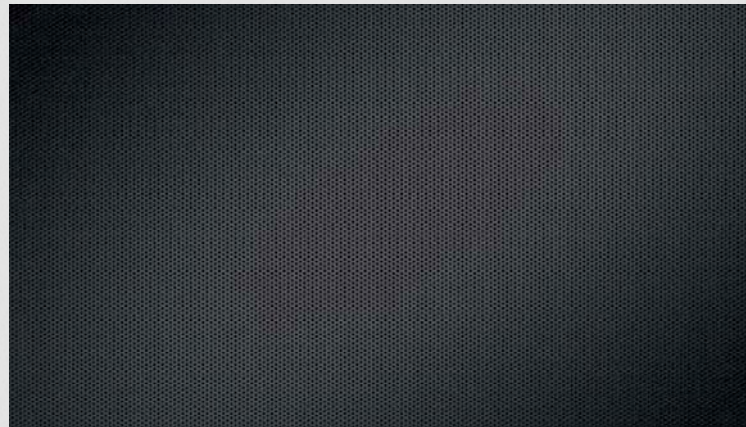


# Day One:

## Teacher Preparation:

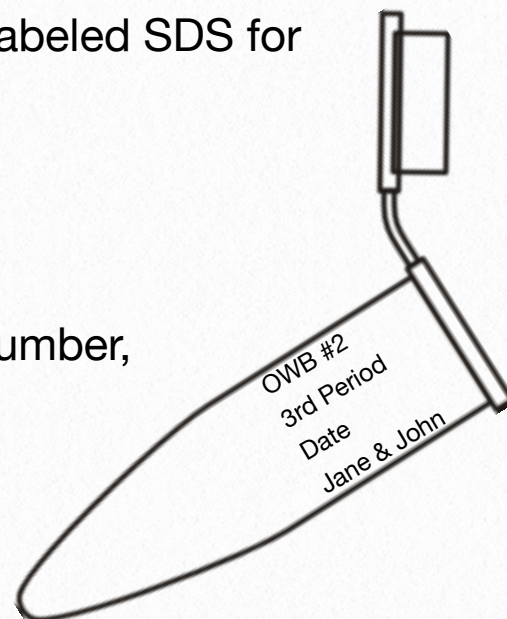
1. Before class, add 400  $\mu$ l **2-Mercaptoethanol** to 19.6 ml of 2x CTAB = 20 ml total. This should be done in a fume hood.
2. Aliquot 805  $\mu$ l of the **2-Mercaptoethanol** and **CTAB** solution into 20 - 1.5 ml microcentrifuge tubes labeled CTAB for students.
3. Aliquot 105  $\mu$ l of **SDS** into 20 - 1.5 ml microcentrifuge tubes labeled SDS for students.

## Movie 2.1 DNA Extraction Day #1



## Student Instructions:

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  $\mu$ l of **CTAB Buffer** and **2-Mercaptoethanol** solution from the tube labeled CTAB and 100  $\mu$ l 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 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.

**\*\* 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. For more information, see the MSDS sheet in Chapter 5 Safety Information.

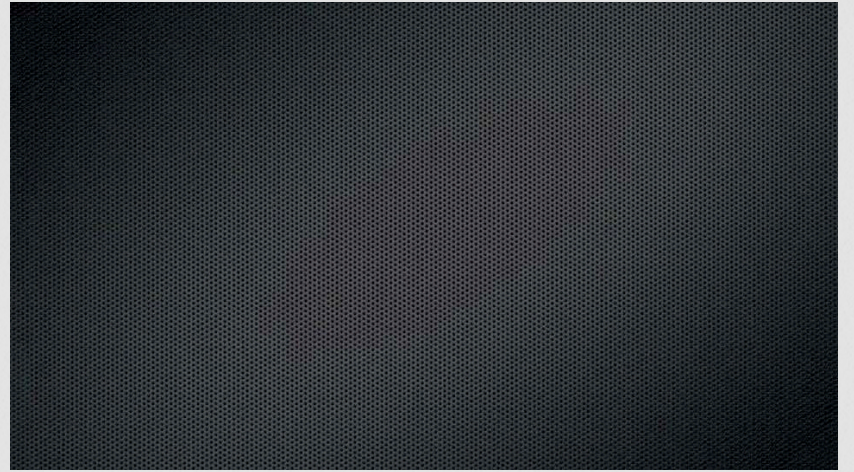


## Day Two:

### Teacher Preparation:

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 needed.

### Movie 2.2 DNA Extraction Day #2



### Student Instructions:

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  $\mu$ l 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).
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  $\mu$ l 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).

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.



## Day Three:

### Teacher Preparation:

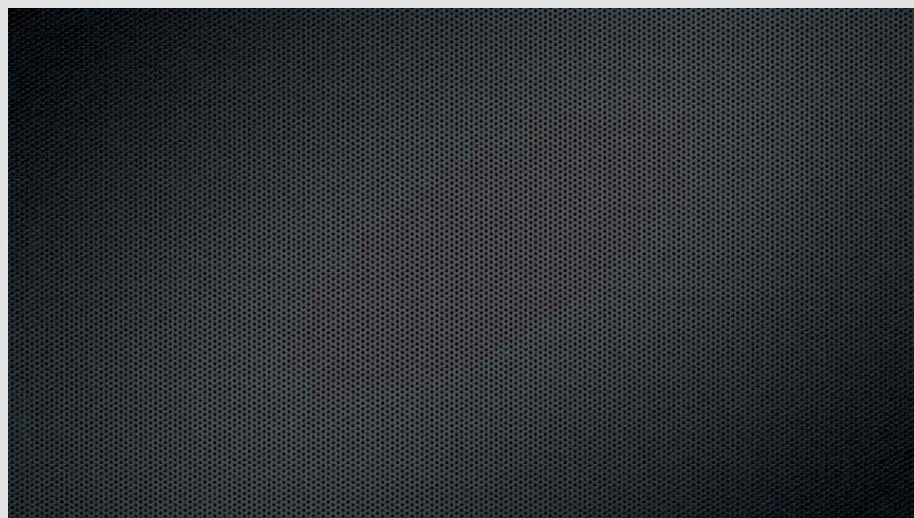
1. Aliquot 505 µl of **70% ethanol** into 20 - 1.5 ml microcentrifuge tubes labeled E for students. This must be kept cold.
2. Aliquot 205 µl of **TE Buffer** into 20 - 1.5 ml microcentrifuge tubes labeled TE for students.

### Student Instructions:

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

#### Movie 2.3 DNA Extraction Day #3





Store these samples overnight at 4°C.

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



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



### 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™ PureTaq™ Ready-To-Go™ PCR Beads  
Store @ Room Temperature



## Materials:

Thermocycler	1.5 ml Centrifuge Tubes	Ice
Micropipettes	Pipette Tips	<i>Kap</i> PCR Primers
Molecular Grade Water	DNA Template(s)	
Regular PCR Tubes Protocol:	0.2 ml PCR Tubes, <b>Taq DNA Polymerase</b> , & <b>Master Mix</b> (see page 16)	

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

## Teacher Preparation: Regular *Taq* Polymerase PCR

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.***

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

## Preparing Primer Mix:

Reagent	Volume ( $\mu$ l per reaction)	Number of Reactions	Total Volume for all reactions
Molecular Grade H <sub>2</sub> O	10.5 $\mu$ l	22	231 $\mu$ l
Master Mix	12.5 $\mu$ l	22	275 $\mu$ l
<i>Kap</i> Primer F	0.5 $\mu$ l	22	11 $\mu$ l
<i>Kap</i> Primer R	0.5 $\mu$ l	22	11 $\mu$ l
<i>Taq</i> Polymerase	0.125 $\mu$ l	22	2.75 $\mu$ l

Total Primer Mix = 530.75  $\mu$ l



## **Teacher Preparation: PCR Beads**

1. 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.***

Reagent	Volume (µl per reaction)	Number of Reactions	Total Volume for all reactions
Molecular Grade H <sub>2</sub> O	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

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

### **Preparing Samples for PCR:**

Total Reaction Volume = 25 µl

### **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)

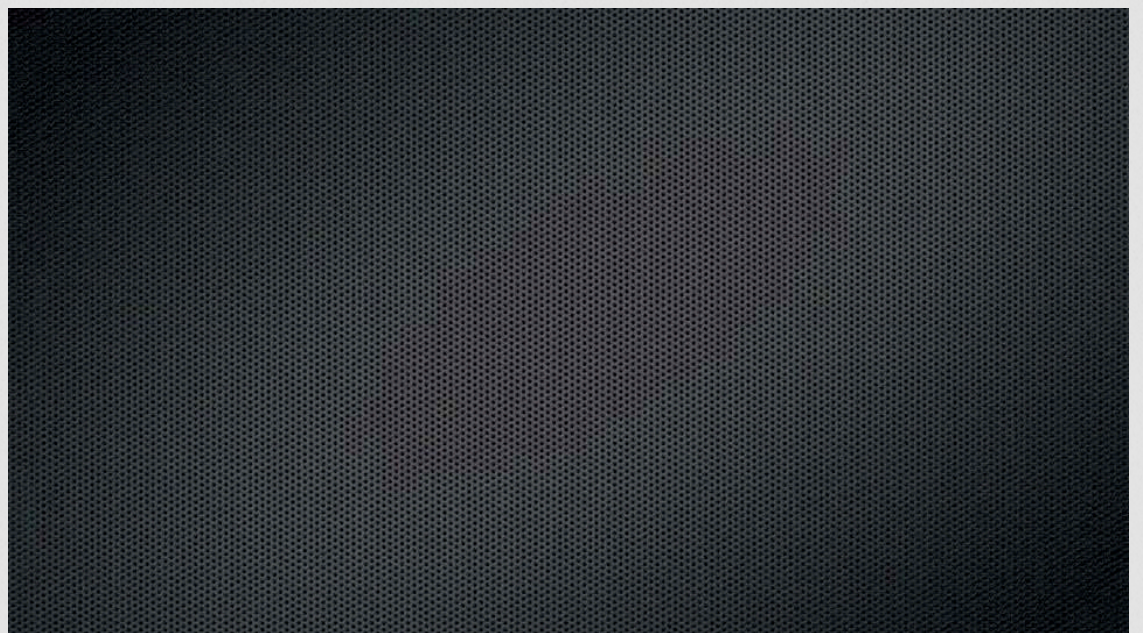


The thermocycler program has a run time of over two hours. Take that into careful consideration when planning for multiple classes running the module.

### **Student Instructions:**

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  $\mu$ l of the hooded/awn (*Kap*) primer mix to your PCR tubes.
4. Add 1  $\mu$ l 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.

#### **Movie 2.4 *Kap* PCR Protocol Demo**



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.



## 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**

### Teacher Preparation - Preparing the Gel:

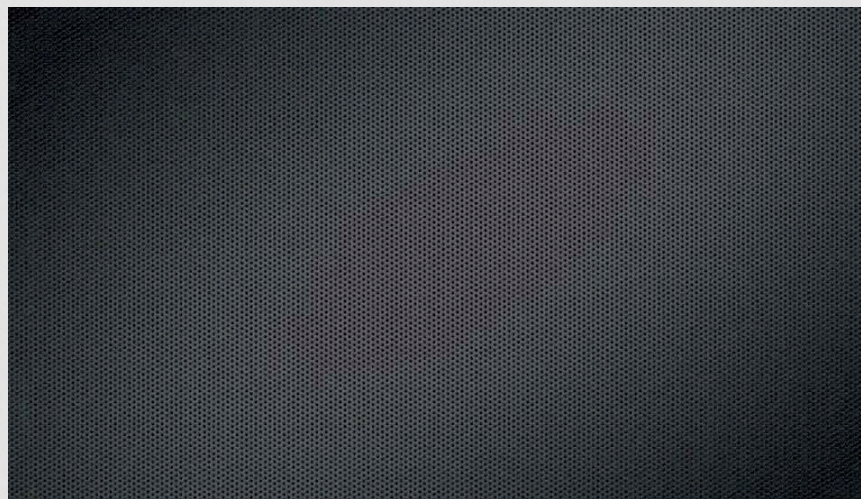
1. First, determine the volume of the gel to be used. This will depend on the length and width of the gel tray, as well as the approximate depth of the gel you want. Multiply the gel volume by 0.01 to determine the number of grams of **agarose** needed for a 1% gel. Using this module, an 80 ml gel will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

\*The 1% gel can be somewhat flimsy. If you want to make a “stiffer” gel, make a 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. 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.)

#### Movie 2.5 Preparing an Electrophoresis Gel



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)

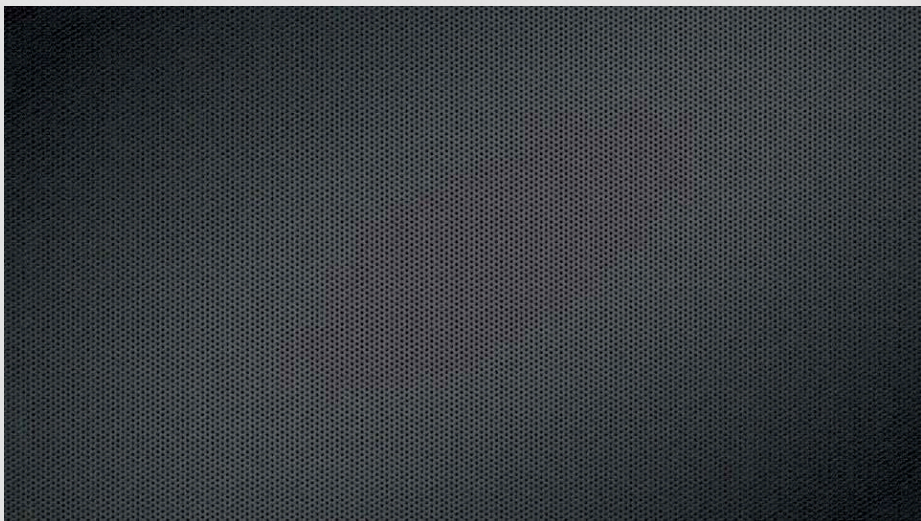
Student Names											
Sample	Ladder	Dom	Rec	2	4	6	9	10	11	13	14
Wells											

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

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

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.
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.

**Movie 2.6 Loading and Running a Gel**





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 may have to run it longer than 30 min.)

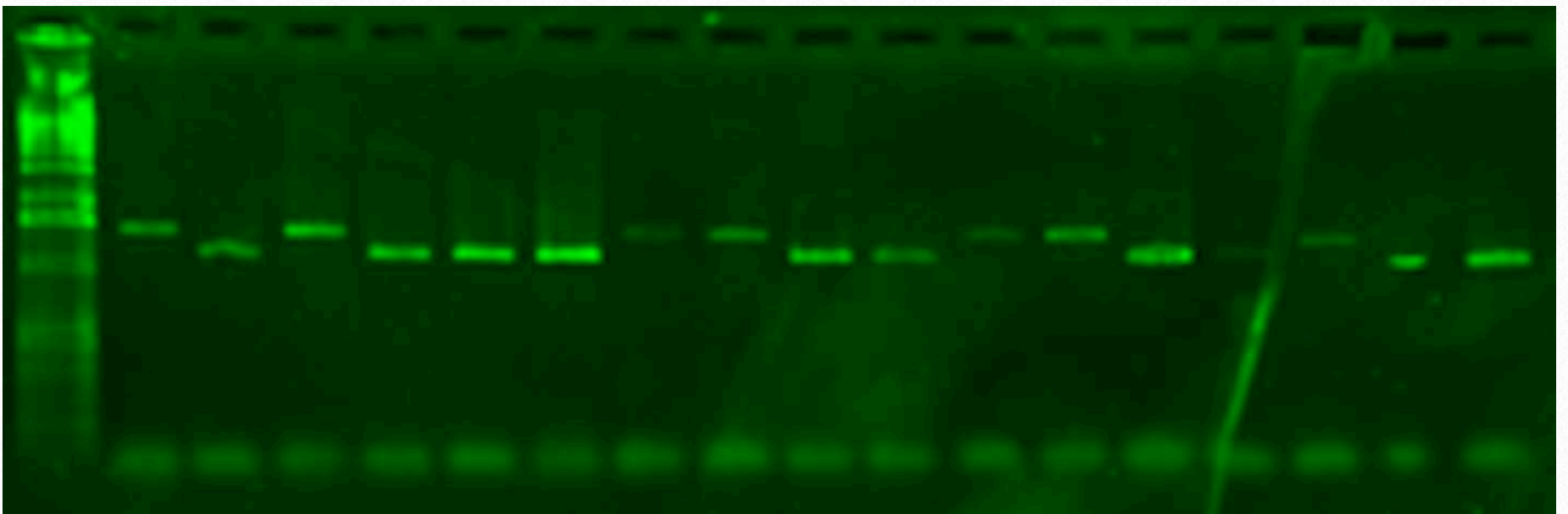
\*\*\* It is important to note: DNA is negatively charged, so it will run from the negative end of the box (black electrode) to the positive end of the box (red electrode). The saying “Run to red” helps to remember.\*\*\*

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.

### **Visualizing the Gel:**

1. The DNA is not visible at this point, but during the electrophoresis the Gel Green stain adhered to 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. The darker the surroundings, the better the bands show up. Turning off the room lights room can help.

An example picture of the gel under the transilluminator:



A picture of a gel of the *Kap* gene with key is included in the Appendix: Answer Key section (Chapter 4, Section 2).



# CHAPTER 3

## Module Extensions





## 3.1 Extensions to the Module

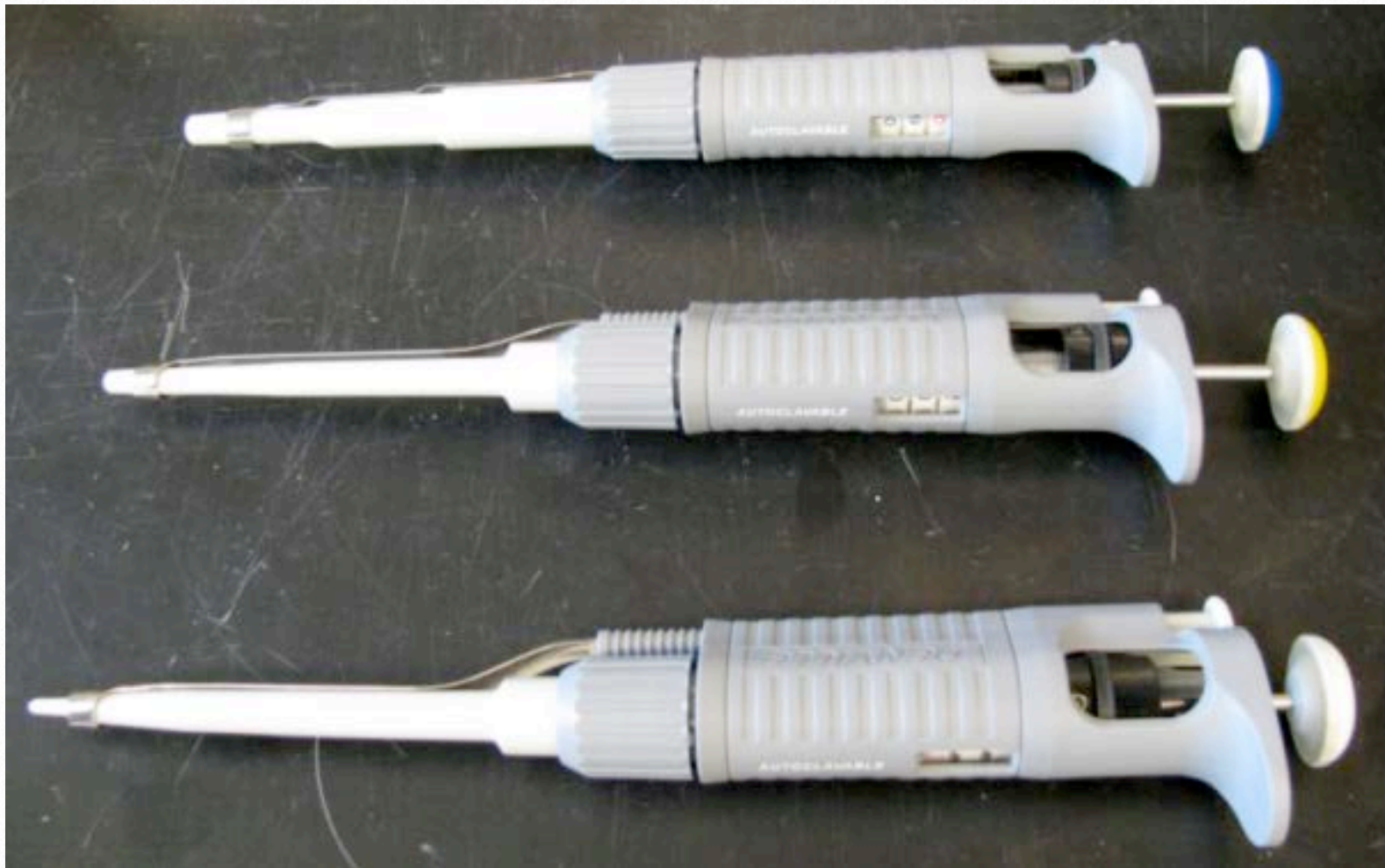
This section includes several optional extension activities for teachers. You may want your students to perform one (or more) of these extension activities in addition to those in the learning module, or you may decide to use them separately in a way that fits your time, curriculum, and equipment needs the best.

The first three activities should be conducted before the Learning Module to provide students with a foundation in basic techniques. **Pipette Practices**(3.2) are useful activities if your students 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 to younger students or to those who have never performed such a procedure before. **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 your students 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 with advanced students or if you have extra time. 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 your students 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 are two different exercises that can be done to help familiarize your students with its proper use. The first involves using an analytical balance to calibrate the micropipettes. The second involves simply practicing adjusting the micropipettes, pipetting set amounts, mixing colored water using the pipettes, and loading the colored water mixtures into a gel to run electrophoresis. (It's a good way to practice loading gels before working with DNA.)

### **Materials Practice #1:**

Micropipettes

Deionized Water

Weigh Boat

Analytical Balance

### **Materials Practice #2:**

Micropipettes

Water

Food Coloring

Microcentrifuge Tubes

Wax Paper/Parafilm

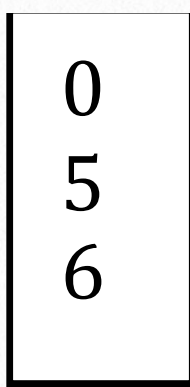
Electrophoresis Gel (Section 2.5)



# Micropipette Practice #1

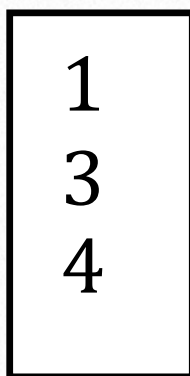
## How to Adjust and Read the Micropipette

It is important for your students 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  $\mu\text{l}$  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,



The micropipette is set to take up and dispense 5.6  $\mu\text{l}$ .

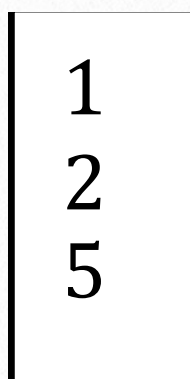
A 2-20  $\mu\text{l}$  micropipette is the same. The top number is the tens place; the middle is the ones place; and the bottom number is the tenths. If your reading looks like this,



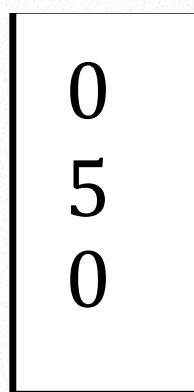
your micropipette is set to transfer 13.4  $\mu\text{l}$ .



Larger pipettes are a bit different. For a 20-200  $\mu\text{l}$  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  $\mu\text{l}$ , you should turn the knob to make the reading look like so:



For a 100-1000  $\mu\text{l}$  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  $\mu\text{l}$ , you should set the micropipette to look like this:

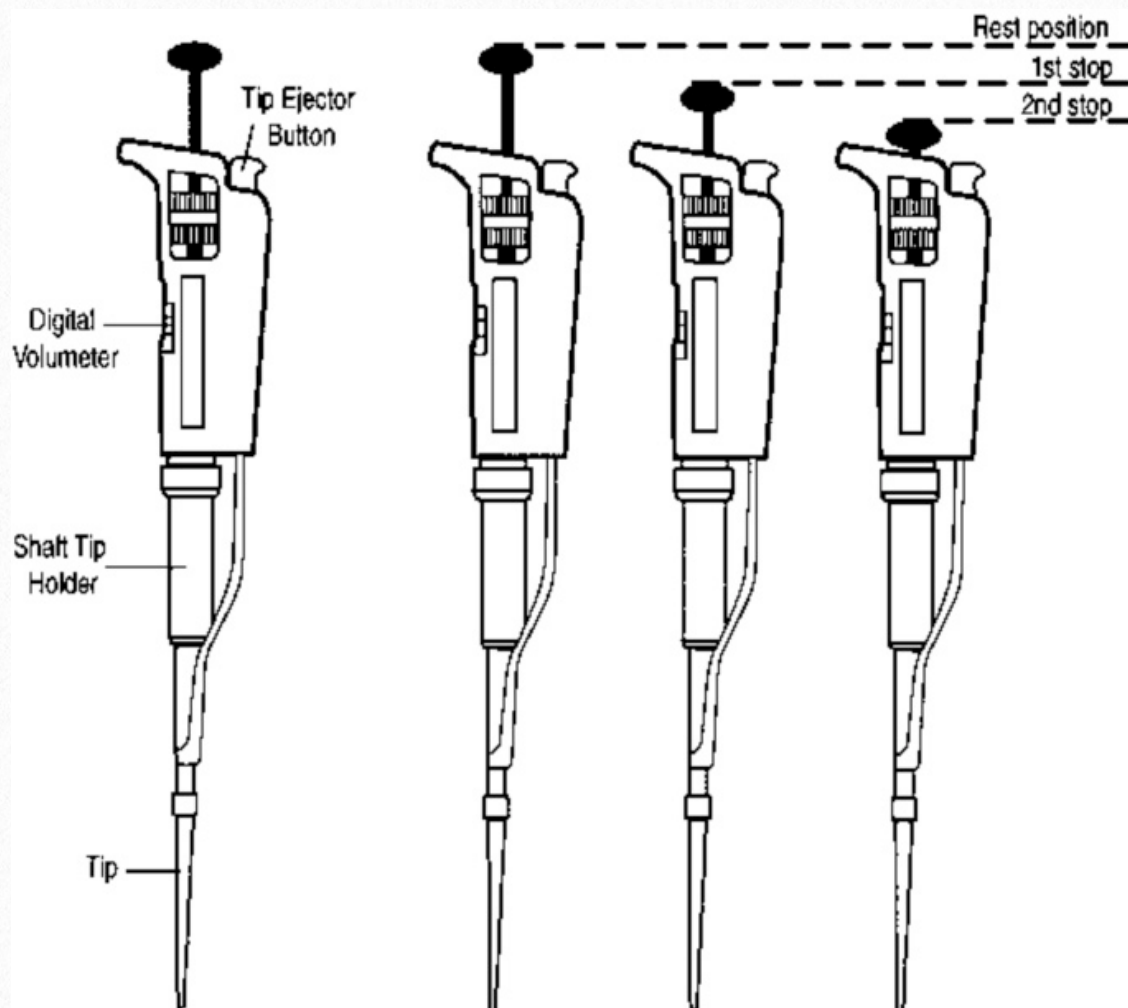


Seem strange? The best way to become familiar with each of the pipettes is to practice setting volumes. You'll get the hang of it quickly. One final note, although a pipette can be dialed above or below its stated range; doing so will throw off the calibration and result in inaccuracy. **NEVER DIAL A PIPETTE ABOVE OR BELOW ITS 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 students 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 ( $1\mu\text{l} = 1\mu\text{g}$ ). 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](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)

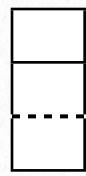
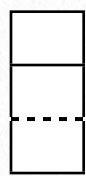
**Precaution:**

- \* 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.\*\**

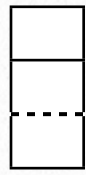
**White Plunger 0.5-10 µl**

2. Set the digital display to the following settings and check with your partner.
- 1.00 µl
- 5.36 µl



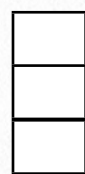
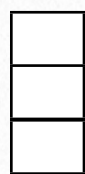
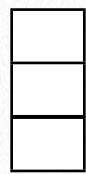
**Yellow Plunger 2-20 µl**

3. Set the digital display to the following settings and check with your partner.
- 6.92 µl
- 15.50 µl



**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





## Blue Plunger 100-1000 $\mu$ l

5. Set the digital display to the following settings and check with your partner.

250  $\mu$ l

----

624  $\mu$ l

----

898  $\mu$ l

----

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

6. You need a volume of 54.5  $\mu$ l. What pipettes would you use to reach this volume?

7. You need a volume of 645.8  $\mu$ l. What pipettes would you use to reach this volume?

8. You need a volume of 1,820.65  $\mu$ l. 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

Styrofoam or Plastic Cups (8 oz.)

Zip-lock Sandwich Bags

Bleached Coffee Filters (white)

95% Ethanol (ice cold)

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  $\frac{1}{4}$  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

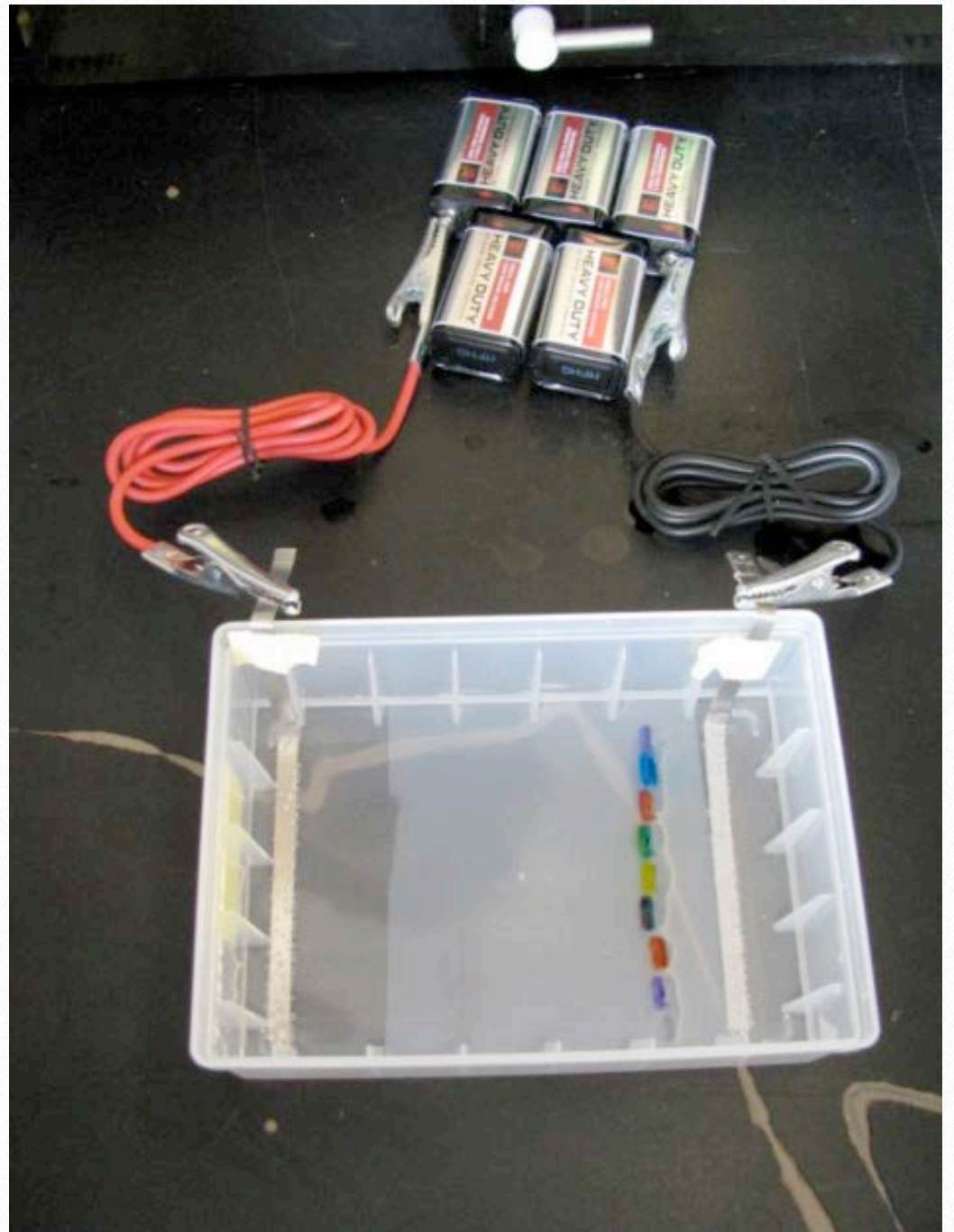
**For the gel and buffer:**

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

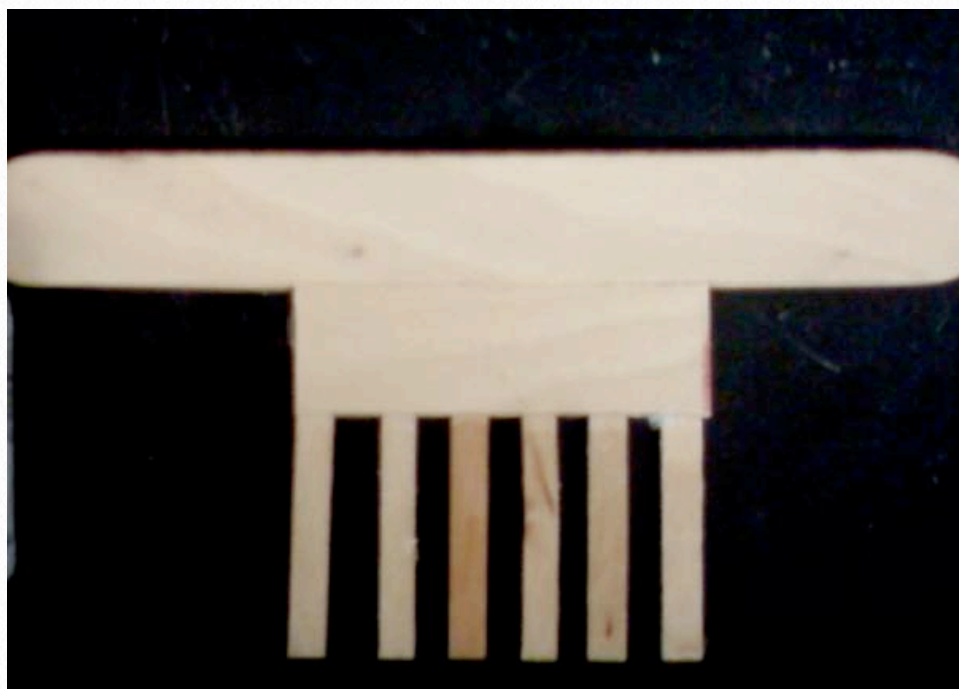
Glycerin (available from pharmacies)

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. Pour 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.

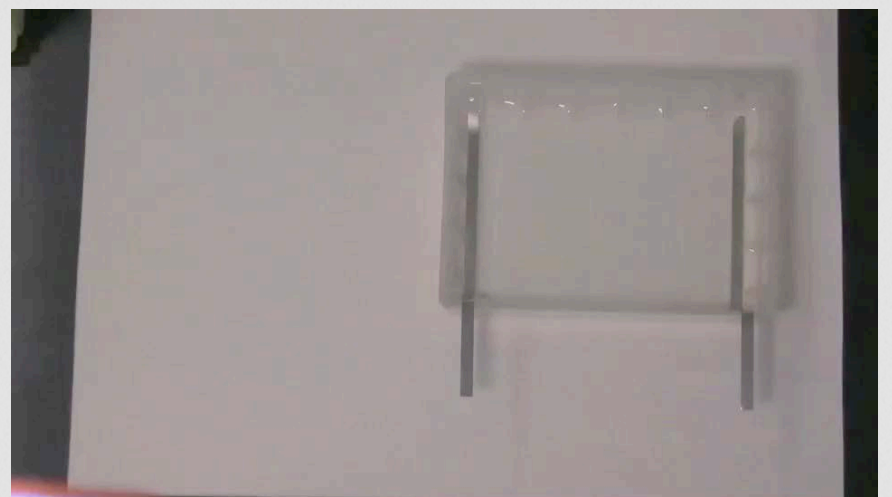


8. Prepare 5 different samples by mixing 1-2 drops of food coloring with 1 ml glycerin and 1 ml water in a small tube. We used blue, red, green, yellow, and purple (made by mixing blue and red food coloring).

## **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  $\mu$ l of each sample to an empty well. The volume of the thin tip of the pipette is about 10  $\mu$ l. 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.

### **Movie 3.1 Tip-Tip Electrophoresis High Speed**





### **Additional Information:**

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

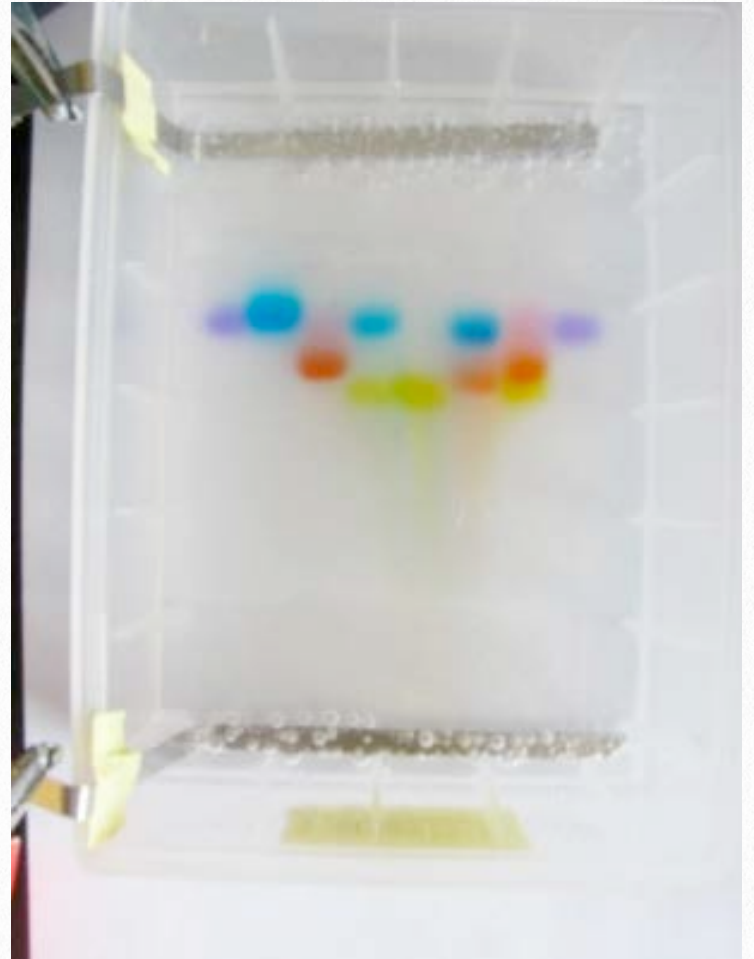
A classic molecular biology text available FREE online at:

<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mcb>

[http://en.wikipedia.org/wiki/Gel\\_electrophoresis](http://en.wikipedia.org/wiki/Gel_electrophoresis)

[http://en.wikipedia.org/wiki/Food\\_dye](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 students 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
Molecular Grade Water	DNA Template(s)	
Regular PCR Tubes Protocol:	0.2 ml PCR Tubes, Taq DNA Polymerase, & Master Mix (see page 16)	

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

**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.**

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.

**Preparing Primer Mix:**

Reagent	Volume (µl per reaction)	Number of Reaction s	Total Volume for all reactions
Molecular Grade H <sub>2</sub> O	10.5 µl	22	231 µl
Master Mix	12.5 µl	22	275 µl
Vrs1 Primer F	0.5 µl	22	11 µl
Vrs1 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 *Vrs1* primers 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 H <sub>2</sub> O	23 µl	22	506 µl
<i>Vrs1</i> Primer F	0.5 µl	22	11 µl
<i>Vrs1</i> Primer R	0.5 µl	22	11 µl

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

Total  
Primer Mix = 528 µl

**Preparing Samples for PCR:**

Total Reaction Volume = 25 µl

**\*\*\*Note the annealing temperature is different than the *Kap* gene protocol.**

**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  $\infty$  (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.

### **Student Instructions:**

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  $\mu$ l of the *Vrs1* primer mix to your PCR tubes.
4. Add 1  $\mu$ l 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.

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™ PureTaq™ Ready-To-Go™ PCR Beads  
Store @ Room Temperature



### 3.6 Restriction Digest of the *Vrs1* Amplicon, Gel Electrophoresis of *Vrs1* PCR Product, Gel Electrophoresis of *Vrs1* Digest Product



#### **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:**

**Teacher Preparation - *Vrs1* Digest:**

1. Create a reaction mix of NEB Buffer 4, *NciI*, and H2O in a 1.5 ml centrifuge tube (see table below). Keep on ice until ready to transfer 5 µl to each student’s tube.

**Restriction Enzyme Digests Reaction Mix:**

Reagent	Volume / Reaction	# of Reactions	Mix Volume
Molecular Grade H <sub>2</sub> O	2.0	22	44
NEB Buffer 4 (10x)	2.5	22	55
<i>NciI</i> (20 µg/µl)	0.5	22	11

Total = 110 µl

**Preparing Samples for Digest:**

Reagent	Volume (µl per reaction)
Reaction Mix	5 µl
Template DNA	20 µl

Reaction Total = 25 µl

**Teacher Preparation: *Vrs1* PCR Product Gel Electrophoresis:**

1. Make a gel using the same instructions from Chapter 2: Section 5.

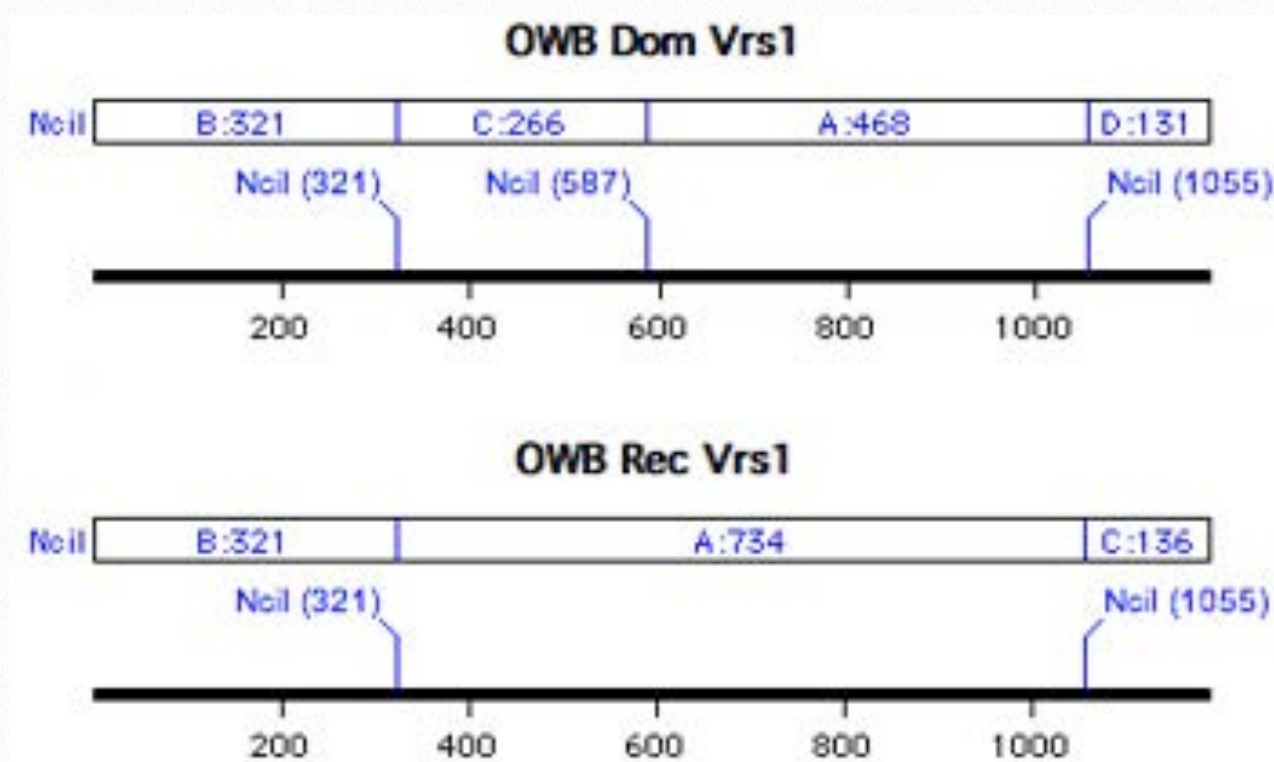
A picture of a gel of the *Vrs1* gene PCR Product with key is included in the Appendix: Answer Key section (Chapter 4, Section 3).



### Student Instructions - *Vrs1* Digest:

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.

### Predicted Fragment Lengths:





### **Student Instructions - *Vrs1* PCR Product Electrophoresis:**

1. On a piece of wax paper (parafilm), combine 1.5  $\mu$ l of **loading dye** with 5  $\mu$ l 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  $\mu$ l 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:**

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

1. Make a gel using the same instructions from Chapter 2: Section 5.

### **Student Instructions: *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, have students visualize the *Vrs1* PCR product gel. A picture of a gel of the *Vrs1* gene PCR Product with key is included in the Appendix: Answer Key section (Chapter 4, Section 3).

## **Day Three:**

### **Teacher Preparation:**

Visualize the gel of the *Vrs1* Digest with students. A picture of a gel of the *Vrs1* gene Digest with key is included in the Appendix: Answer Key section (Chapter 4, Section 3).



# Restriction Digest Modeling Activity



Students often have difficulty understanding the restriction enzyme length polymorphisms of the amplified DNA of the *Vrs1* gene. The following activity can be done with students to help this abstract concept become more concrete with hands-on modeling.

Learn.Genetics through the University of Utah has a great virtual lab available that explains how fragments of DNA travel through a gel.

<http://learn.genetics.utah.edu/content/labs/gel/>

## **Materials:**

Paper copies of dominant and recessive *Vrs1* alleles

Blank gel template

Scissors

Here is a link to a Google Document of this activity you can edit as needed.

[Modeling Activity Document Link](#)



## **Overview**

Gel electrophoresis is a process where the different fragments of DNA in a sample are sorted according to their length. The gel acts like a filter, where the DNA fragments travel through the many small holes in the gel. Samples are loaded in the wells at one end of the gel and then a current is added. DNA is slightly negatively charged, so it will travel from the negative electrode (colored black) to the positive electrode (colored red).

Short DNA fragments will travel faster through the tiny holes in the gel, while the longer DNA fragments will travel slower. DNA fragments that are the same length travel at the same speed. At the end of the “run time” the fragments will have separated allowing you to find their approximate base pair length. Learn.Genetics through the University of Utah has a great virtual lab available that explains how fragments of DNA travel through a gel.

<http://learn.genetics.utah.edu/content/labs/gel/>

## **Student Instructions**

1. Open the envelope and remove the red strip of paper representing dominant *Vrs1* allele and the blue strip of paper representing the recessive *vrs1* allele.
2. Use the scissors to represent the enzyme and cut (digest) each allele along the black lines.
3. Arrange the digested fragments on the gel electrophoresis template according to their base pair lengths. The red dominant fragments should be in one lane, while the blue recessive fragments should be in the other lane.

## **Questions**

1. How many bands are there for the dominant *Vrs1* allele? The recessive *Vrs1* allele?
2. What caused the individual alleles to be digested (cut)?
3. Why do some DNA fragments move farther in the gel than others?
4. Why do the *Vrs1* alleles require a digest using a restriction enzyme and the *Kap* alleles did not?





In barley varieties, the *Vrs1* dominant allele codes for a phenotype called Two-Row seed arrangement, while the *vrs1* recessive allele codes for a phenotype called Six-Row seed arrangement.



*Ncil* enzyme digests (cuts) alleles along the black lines.



In barley varieties, the *Vrs1* dominant allele codes for a phenotype called Two-Row seed arrangement, while the *vrs1* recessive allele codes for a phenotype called Six-Row seed arrangement.



*Ncil* enzyme digests (cuts) alleles along the black lines.



In barley varieties, the *Vrs1* dominant allele codes for a phenotype called Two-Row seed arrangement, while the *vrs1* recessive allele codes for a phenotype called Six-Row seed arrangement.



*Ncil* enzyme digests (cuts) alleles along the black lines.



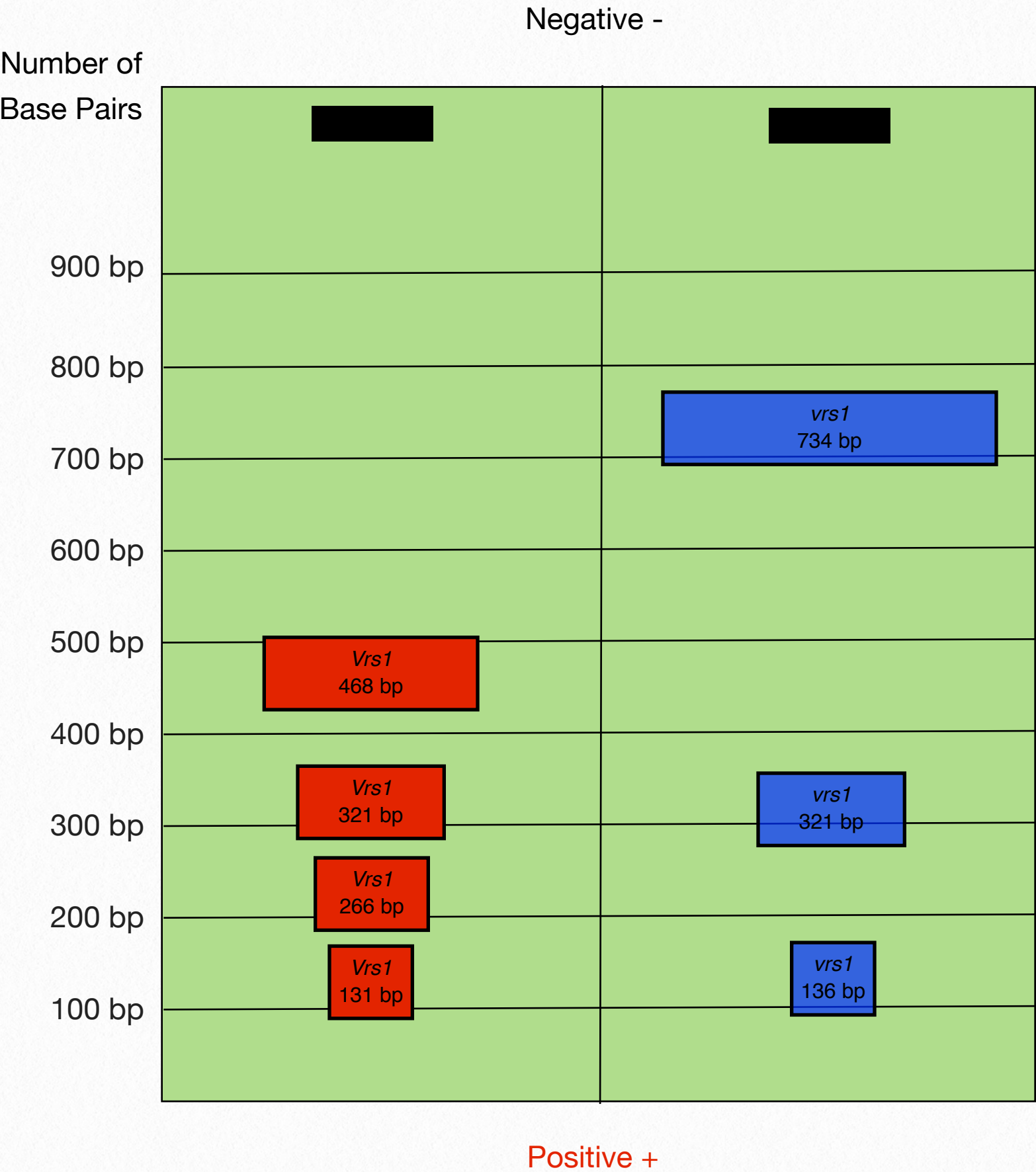
# Gel Electrophoresis Template

Negative -	
Number of Base Pairs	
900 bp	
800 bp	
700 bp	
600 bp	
500 bp	
400 bp	
300 bp	
200 bp	
100 bp	
Positive +	



Key

Gel Electrophoresis Template





# CHAPTER 4

## Appendix

**iTAG Barley**

[resistancegenomics.org](http://resistancegenomics.org)





## 4.1 Stock Solutions

### **2X CTAB Buffer:**

### **Per 500 ml**

Tris-HCl	100 mM
NaCl	1.4 M
EDTA (Ethylenediamine-tetraacetic acid)	20 mM
CTAB (Hexadecyltriethyl-ammonium bromide)	10 g
2-Mercaptoethanol (added the day of extraction)	400 $\mu$ l

### **1X TE Buffer:**

### **Per 100 ml**

10 mM Tris-HCl (pH 8.0) (Use 1M stock)	1.0 ml
1 mM EDTA (Use 0.5M pH 8.0 stock)	50 $\mu$ l

### **10X TBE Buffer:**

### **Per 1000 ml**

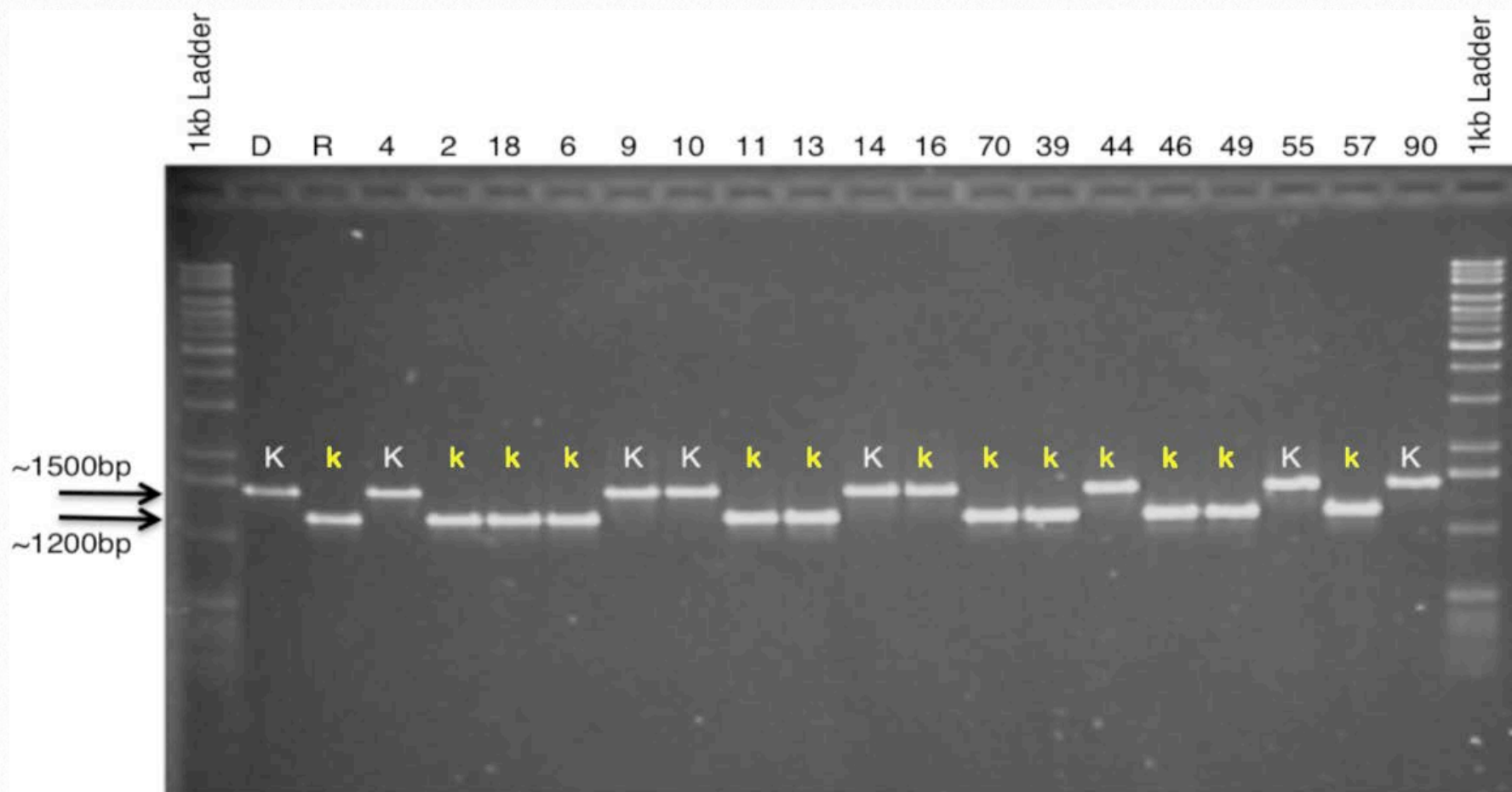
0.89 M Tris	108 g
0.89 M Boric Acid	55 g
0.02 M EDTA	9.3 g

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



## 4.2 Answer Key: *Kap* Amplification

### *Kap* Amplicon Gel Key – OWB segregating double haploid progeny



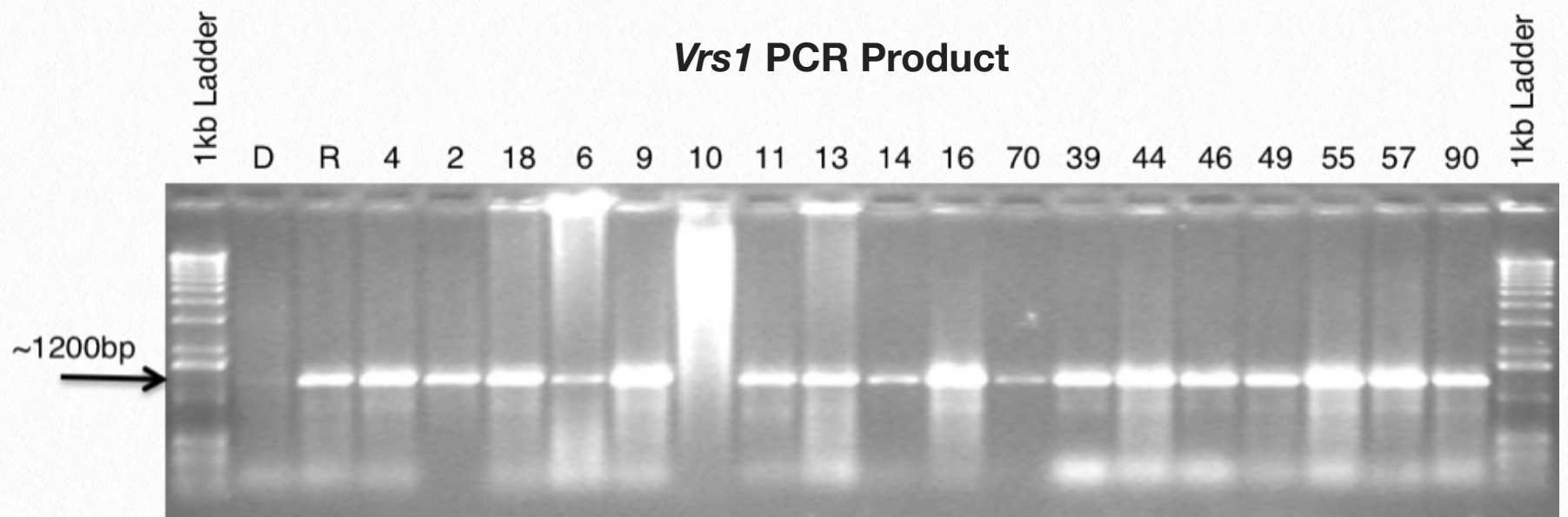
The characters above each well represent the parents (D for Dominant and R for Recessive), and the 18 lines of the OWB segregating **double haploid** progeny. The row of letters above the bands indicates the phenotype; K stands for the dominant “hooded” phenotype while k stands for the recessive “Awn” phenotype. The bands represent the DNA fragment amplified during PCR. The hooded phenotype has a 1500 bp band while the awn phenotype has a 1200 bp band.

Lines 16 and 44 have the 1500 bp band but show the awn phenotype. This is due to **epistasis** of the **Kap gene by another gene named Lks2**. A dominant *Lks2* allele must be present for the hooded phenotype to occur. When a recessive *Lks2* allele is present with a dominant *Kap* allele, the plant will have the awn phenotype. This is the case in lines 16 and 44.



## 4.3 Answer Key: *Vrs1* PCR Gel

*Vrs1* Amplicon Gel Key – OWB segregating double haploid progeny



Unlike the *Kap* amplicons, the *Vrs1* amplicons are not size **polymorphic** as seen in the previous gel. The difference in sequence can be visualized using the **NciI restriction enzyme**. This enzyme's restriction site is present twice in the recessive allele(r) and three times in the dominant allele(R)

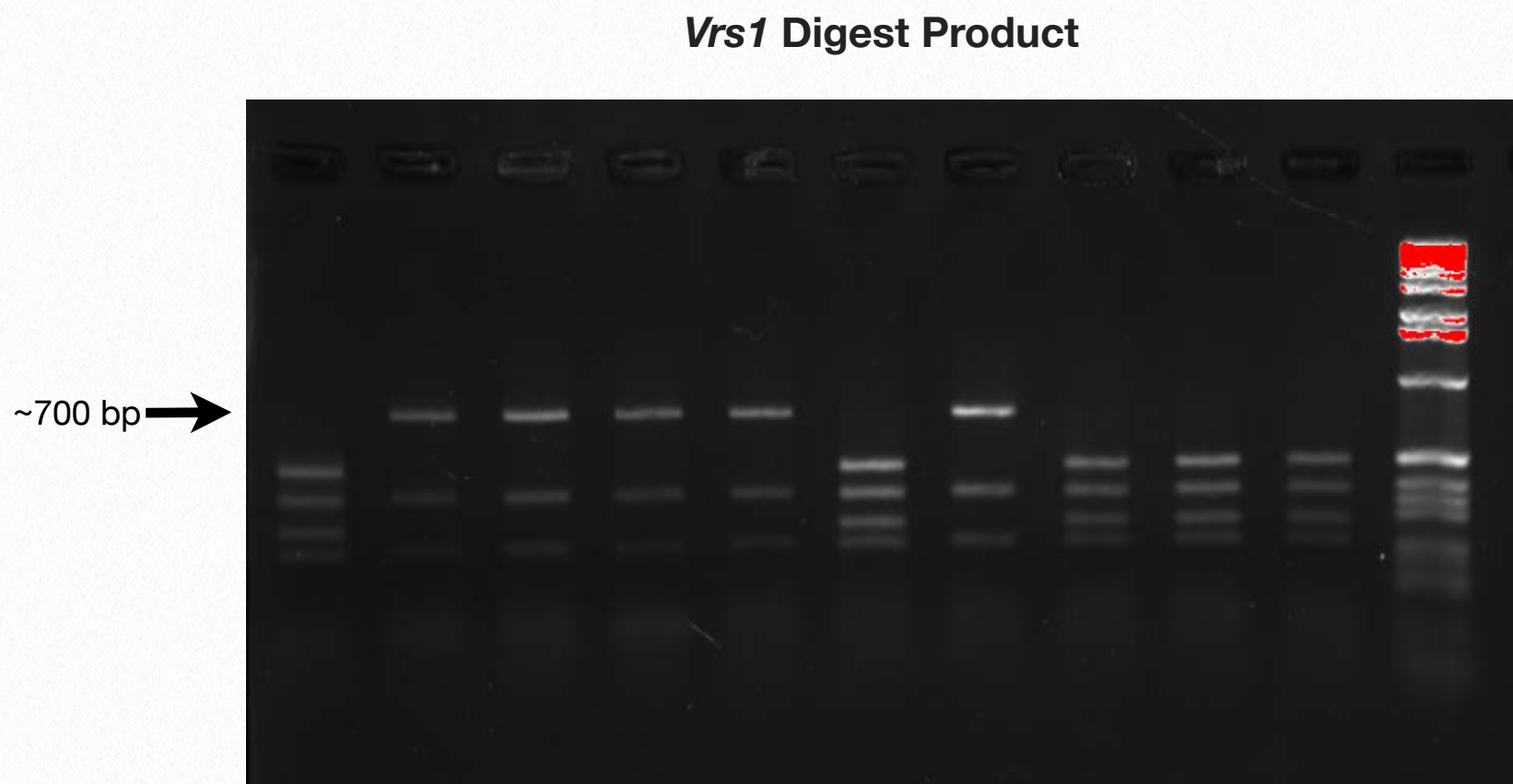


## 4.4 Answer Key: *Vrs1* Digestion Gel



The letters below each lane represent the phenotype of each individual where R refers to a **two-row seed spike** and r refers to a **six-row seed spike**. After digestion with *NciI*, the 1200 bp amplicon is cut into four pieces in the dominant allele and three pieces in the recessive allele. The 700 bp band of the recessive allele is most obvious as it sits well above the smaller fragments.

Another picture of the *Vrs1* Digest Gel showing the four bands in the dominant allele and three bands in the recessive allele (distinct band at 700 bp).





## 4.4 Iowa Core Standards

Standard	Benchmark	Action
Unifying concepts and processes in science	Evidence, models, and explanation	Students will use evidence they gather, both observationally and experimentally, to explain why some plants have trait A while other plants have trait B.
Science as Inquiry	Understanding of scientific concepts	The focus of this module is on understanding cosegregation of genotypes and phenotypes, not on memorizing terms like <i>genotype</i> and <i>phenotype</i> .
	An appreciation of “how we know” what we know in science	Students will experience “how we know” that genotypes determine phenotypes.
	Understanding of the nature of science	This module demonstrates many facets of the nature of science including, but not limited to, science takes time, experiments don’t always work the way in which they are expected, data is interpreted, etc.
	Skills necessary to become independent inquirers about the natural world	The thought processes developed in this module are able to be applied to all scientific endeavors.
	The dispositions to use the skills, abilities, and attitudes associated with science	Students should collaborate, use scientific language, base arguments on data and evidence, make observations, draw conclusions and write a lab report.
Life Science Standards	Molecular basis of heredity	This is the foundation of the iTAG Barley Gene Expression and Segregation Analysis module.
	Biological evolution	Variation in organisms is heritable.
Science and Technology	Understanding about science and technology	The use of technology is not always required but often enables deeper understanding of fundamental concepts. Tip Top electrophoresis provides a simple scaffold for gel electrophoresis of PCR amplicons.
Science in Personal and Social Perspectives	Science and technology in local, national, and global challenges	Iowa’s economy is largely based on agriculture. Our nation is attempting to reduce dependence on foreign oil and develop more environmentally friendly sources of energy. The world’s population is rapidly increasing which requires much greater food production from the same size or smaller plots of land.
History and Nature of Science	Science as a human endeavor	Students will build understanding based on their own observations and evidence. This experience can be broadened to explain the development of all scientific understanding.
	Nature of scientific knowledge	What is known is based only on the available data and its interpretation.



## 4.5 Next Generation Science Standards

Next Generation Science Standard	Performance Expectation	Foundation from <i>A Framework for K-12 Science Education</i>	Common Core Standards Connections
From Molecules to Organisms: Structures and Processes	HS-LS-1 Construct an explanation based on evidence for how the structure of DNA determines the structure of protein which carry out the essential functions of life through systems of specialized cells.	<b>Science and Engineering Practices:</b> Constructing Explanations and Designing Solutions <b>Disciplinary Core Ideas:</b> LS1.A Structure and Function <b>Crosscutting Concepts:</b> Structure and Function	<b>ELA/Literacy:</b> RST-11.12.1 Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account. WHST.9-12.5 Write informative /explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes. WHST.9-12.9 Draw evidence from informational texts to support analysis, reflection, and research.
Heredity: Inheritance and Variation of Traits	HS-LS3-1 Ask questions to clarify relationships about the role DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	<b>Science and Engineering Practices:</b> Asking Questions and Defining Problems <b>Disciplinary Core Ideas:</b> LS1.A Structure and Function, LS3.A Inheritance of Traits <b>Crosscutting Concepts:</b> Cause and Effect	<b>ELA/Literacy:</b> RST-11.12.1 Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account. RST-11.12.9 Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.
Biological Evolution: Unity and Diversity	HS-LS4-1 Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.	<b>Science and Engineering Practices:</b> Obtaining, Evaluation, and Communicating Information Connections to Nature of Science: Science Models, Laws, Mechanisms, and Theories Explain Natural Phenomena <b>Disciplinary Core Ideas:</b> LS4.A Evidence of Common Ancestry and Diversity <b>Crosscutting Concepts:</b> Patterns Connections to Nature of Science: Scientific Knowledge Assumes an Order and Consistency in Natural Systems	<b>ELA/Literacy:</b> RST-11.12.1 Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account. WHST.9-12.2 Write informative / explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes. WHST.9-12.9 Draw evidence from informational texts to support analysis, reflection, and research. SL.11-12.4 Present claims and findings, emphasizing salient points in a focused, coherent manner with relevant evidence, sound valid reasoning, and well-chosen details; use appropriate eye contact, adequate volume, and clear pronunciation. <b>Mathematics:</b> MP.2



iTAG Barley Growth Chart  
Period

OWB #	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
DOM										
REC										
#2										
#4										
#6										
#9										
#10										
#11										
#13										
#14										
#16										
#18										
#39										
#44										
#46										
#49										
#55										
#57										
#70										
#90										



# 4.6 Acknowledgements

## Protocols

### 1 – Growing Instructions for Oregon Wolfe Barley

<http://barleyworld.org/oregonwolfe>

Modified from protocol at [www.barleyworld.org](http://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

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.

### 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](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**

Modified from Sandra Slutz, PhD, Science Buddies, [http://www.sciencebuddies.org/science-fair-projects/project\\_ideas/BioChem\\_p028.shtml](http://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p028.shtml)

## **8 – Polymerase Chain Reaction of *Vrs1* gene**

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

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.

## **9 – Restriction Digest of *Vrs1* Amplicon**

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

## **10 – Restriction Digest Modeling Activity**

Protocol by Ron Schuck, Ames High School, Ames, IA

## **11 – Fun videos:**

[http://www.youtube.com/watch?NR=1&v=khBmRuFc\\_P4](http://www.youtube.com/watch?NR=1&v=khBmRuFc_P4)

<http://www.youtube.com/watch?v=lpMOrX1fzGM&feature=related>

## **12 – 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.



# CHAPTER 5

## Safety Information

**iTAG Barley**

resistancegenomics.org



### SIGMA-ALDRICH

#### 1. PRODUCT AND COMPANY IDENTIFICATION

[sigma-aldrich.com](http://sigma-aldrich.com)

### SAFETY DATA SHEET

Version 5.5 Revision Date 04/16/2015 Print Date 07/09/2015

#### 1.1 Product identifiers

Product name :

**2-Mercaptoethanol**

Product Number Brand

CAS-No.

: M6250 : Aldrich

: 60-24-2

#### 1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

#### 1.3 Details of the supplier of the safety data sheet

Company :

Telephone : Fax :

#### 1.4 Emergency telephone number

Emergency Phone # :

Sigma-Aldrich  
3050 Spruce Street SAINT LOUIS MO 63103 USA

+1 800-325-5832 +1 800-325-5052

(314) 776-6555

#### 2. HAZARDS IDENTIFICATION

##### 2.1 Classification of the substance or mixture GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Flammable liquids (Category 4), H227

Acute toxicity, Oral (Category 3), H301

Acute toxicity, Inhalation (Category 3), H331

Acute toxicity, Dermal (Category 2), H310

Skin irritation (Category 2), H315

Serious eye damage (Category 1), H318

Skin sensitisation (Category 1), H317

Specific target organ toxicity - repeated exposure, Oral (Category 2), Liver, Heart, H373 Acute aquatic toxicity (Category 1), H400

Chronic aquatic toxicity (Category 1), H410

For the full text of the H-Statements mentioned in this Section, see Section 16.

##### 2.2 GHS Label elements, including precautionary statements



Pictogram

Signal word

Hazard statement(s) H227

H301 H310 H315 H317 H318

Aldrich - M6250

Danger

Combustible liquid.

Toxic if swallowed or if inhaled Fatal in contact with skin.

Causes skin irritation.

May cause an allergic skin reaction. Causes serious eye damage.



+ H331

Page 1 of 9



H373

H410

Precautionary statement(s) P210  
P260  
P262

May cause damage to organs (Liver, Heart) through prolonged or repeated exposure if swallowed.  
Very toxic to aquatic life with long lasting effects.

Keep away from heat/sparks/open flames/hot surfaces. - No smoking. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.  
Do not get in eyes, on skin, or on clothing.  
Wash skin thoroughly after handling.

Do not eat, drink or smoke when using this product.  
Use only outdoors or in a well-ventilated area.  
Contaminated work clothing should not be allowed out of the workplace. Avoid release to the environment.  
Wear protective gloves/ protective clothing/ eye protection/ face protection.  
IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician. Rinse mouth.  
IF ON SKIN: Gently wash with plenty of soap and water. Immediately call a POISON CENTER or doctor/ physician.  
IF ON SKIN: Wash with plenty of soap and water.  
IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or doctor/ physician.  
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.  
Get medical advice/ attention if you feel unwell.  
If skin irritation or rash occurs: Get medical advice/ attention.  
Take off contaminated clothing and wash before reuse.  
In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.  
Collect spillage.  
Store in a well-ventilated place. Keep container tightly closed.  
Store in a well-ventilated place. Keep cool.  
Store locked up.  
Dispose of contents/ container to an approved waste disposal plant.

P264 P270 P271 P272 P273 P280

. P301 + P310

. P302 + P350

P302 + P352

. P304 + P340

. P305 + P351

P314  
P333 + P313 P362  
P370 + P378

P391  
P403 + P233 P403 + P235 P405  
P501

+ P330 + P310

+ P311  
+ P338 + P310

**2.3 Hazards not otherwise classified (HNOC) or not covered by GHS**

Stench.  
Stench., Rapidly absorbed through skin.

**3. COMPOSITION/INFORMATION ON INGREDIENTS 3.1 Substances**

Synonyms

Formula

Molecular weight CAS-No.  
EC-No.

Aldrich - M6250

: Thioethylene glycol 2-Hydroxyethylmercaptan BME  
β-Mercaptoethanol

: C<sub>2</sub>H<sub>6</sub>OS

: 78.13 g/mol : 60-24-2  
: 200-464-6

**Hazardous components**

Component	Classification	Concentration
2-Mercaptoethanol	Flam. Liq. 4; Acute Tox. 3; Acute Tox. 2; Skin Irrit. 2; Eye Dam. 1; Skin Sens. 1; STOT RE 2; Aquatic Acute 1; Aquatic Chronic 1; H227, H301 +	<= 100 %

Page 2 of 9

H331, H310,  
H315, H317,  
H318, H373,  
H410

For the full text of the H-Statements mentioned in this Section, see Section 16.

**4. FIRST AID MEASURES**

**4.1 Description of first aid measures**

**General advice**  
Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

**If inhaled**  
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

**In case of skin contact**



Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

**In case of eye contact**

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. Continue rinsing eyes during transport to hospital.

**If swallowed**

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

No data available

**5. FIREFIGHTING MEASURES**

**5.1 Extinguishing media**

**Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

Carbon oxides, Sulphur oxides

**5.3 Advice for firefighters**

Wear self-contained breathing apparatus for firefighting if necessary.

**5.4 Further information**

Use water spray to cool unopened containers.

**6. ACCIDENTAL RELEASE MEASURES**

**6.1 Personal precautions, protective equipment and emergency procedures**

Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas. For personal protection see section 8.

**6.2 Environmental precautions**

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

**6.3 Methods and materials for containment and cleaning up**

Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13). Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.


**7. HANDLING AND STORAGE**

**7.1 Precautions for safe handling**

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.

Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge. For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Recommended storage temperature 2 - 8 °C

Storage class (TRGS 510): Non-combustible, acute toxic Cat. 1 and 2 / very toxic hazardous materials

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

**8. EXPOSURE CONTROLS/PERSONAL PROTECTION**

**8.1 Control parameters**

**Components with workplace control parameters**

**8.2 Exposure controls**

**Appropriate engineering controls**

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

**Personal protective equipment**

Component	CAS-No.	Value	Control parameters	Basis
2-Mercaptoethanol	60-24-2	TWA	0.20000 0 ppm	USA. Workplace Environmental Exposure Levels (WEEL)
	Remarks	Skin		

Aldrich - M6250

**Eye/face protection**

Tightly fitting safety goggles. Faceshield (8-inch minimum). Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

**Skin protection**

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.



Full contact  
Material: butyl-rubber  
Minimum layer thickness: 0.3 mm  
Break through time: 480 min  
Material tested: Butoject® (KCL 897 / Aldrich Z677647, Size M)

Splash contact  
Material: Nature latex/chloroprene  
Minimum layer thickness: 0.6 mm  
Break through time: 30 min  
Material tested: Lapren® (KCL 706 / Aldrich Z677558, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374  
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

## Body Protection

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Page 4 of 9

## Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

## Control of environmental exposure

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

- 
- . a) Appearance
  - . b) Odour
  - . c) Odour Threshold
  - . d) pH
  - . e) Melting point/freezing point
  - . f) Initial boiling point and boiling range
  - . g) Flash point
  - . h) Evaporation rate

- . i) Flammability (solid, gas)
- . j) Upper/lower flammability or explosive limits
- . k) Vapour pressure
- . l) Vapour density
- . m) Relative density
- . n) Water solubility
- . o) Partition coefficient: n- octanol/water
- . p) Auto-ignition temperature
- . q) Decomposition temperature
- . r) Viscosity
- . s) Explosive properties
- . t) Oxidizing properties

### 9.2 Other safety information

Relative vapour density

## 10. STABILITY AND REACTIVITY 10.1 Reactivity

No data available

### 10.2 Chemical stability

Form: liquid  
Colour: colourlessyellow

Stench.  
No data available  
4.5 - 6 at 500 g/l at 20 °C (68 °F) < -49.99 °C (< -57.98 °F)

157 °C (315 °F) - lit.

74 °C (165 °F) - closed cup

No data available

No data available

Upper explosion limit: 18 %(V) Lower explosion limit: 2.3 % (V)

0.76 hPa (0.57 mmHg) at 20 °C (68 °F) 4.67 hPa (3.50 mmHg) at 40 °C (104 °F)

2.70 - (Air = 1.0)  
1.114 g/cm<sup>3</sup> at 25 °C (77 °F)  
soluble  
log Pow: -0.326log Pow: -0.056 at 25 °C (77 °F)

No data available No data available

No data available No data available No data available

2.70 - (Air = 1.0)

---

Stable under recommended storage conditions. Aldrich - M6250



. **10.3 Possibility of hazardous reactions**

No data available

. **10.4 Conditions to avoid**

Heat, flames and sparks.

. **10.5 Incompatible materials**

Metals, Oxidizing agents

. **10.6 Hazardous decomposition products**

Other decomposition products - No data available In the event of fire: see section 5

**11. TOXICOLOGICAL INFORMATION**

**11.1 Information on toxicological effects**

**Acute toxicity**

LD50 Oral - Rat - 98 - 162 mg/kg (OECD Test Guideline 401)

LC50 Inhalation - Rat - 4 h - 2 mg/l LC50 Inhalation - Rat - 4 h - 625 ppm LD50 Dermal - Rabbit - 112 mg/kg No data available

**Skin corrosion/irritation**

Skin - Rabbit

Result: Irritating to skin. (Draize Test)

**Serious eye damage/eye irritation**

Eyes - Rabbit

Result: Risk of serious damage to eyes.

**Respiratory or skin sensitisation**

Maximisation Test (GPMT) - Guinea pig May cause sensitisation by skin contact. (OECD Test Guideline 406)

**Germ cell mutagenicity**

Experiments showed mutagenic effects in cultured bacterial cells.

**Carcinogenicity**

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

**Reproductive toxicity**

No data available

No data available

**Specific target organ toxicity - single exposure**

No data available

**Specific target organ toxicity - repeated exposure**

Ingestion - May cause damage to organs through prolonged or repeated exposure. - Liver, Heart Aldrich - M6250

Page 6 of 9

---

**Aspiration hazard**

No data available

**Additional Information**

RTECS: KL5600000

burning sensation, Cough, wheezing, laryngitis, Shortness of breath, Headache, Nausea, Vomiting, Weakness, Unconsciousness, Material is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin., spasm, inflammation and edema of the larynx, spasm, inflammation and edema of the bronchi, pneumonitis, pulmonary edema

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

**12. ECOLOGICAL INFORMATION 12.1 Toxicity**

---

Toxicity to fish

Toxicity to daphnia and other aquatic invertebrates

Toxicity to algae

Toxicity to bacteria

LC50 - Leuciscus idus (Golden orfe) - 46 - 100 mg/l - 96.0 h

EC50 - Daphnia (water flea) - 0.89 mg/l - 48 h (OECD Test Guideline 202)

EC50 - Desmodesmus subspicatus (green algae) - 12 mg/l - 72 h

LC50 - Bacteria - 125 mg/l - 17 h

**12.2 Persistence and degradability**

Biodegradability

Biochemical Oxygen Demand (BOD)

Chemical Oxygen Demand (COD)

Result: < 30.0 % - Not readily biodegradable. Result: 6 % - Not readily biodegradable. aerobic - Exposure time 28 d Result: < 10 % - Not readily biodegradable.

105 mg/g

1.894 mg/g

. **12.3 Bioaccumulative potential**

Does not accumulate in organisms.



. **12.4 Mobility in soil**

No data available

. **12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

. **12.6 Other adverse effects**

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal. Very toxic to aquatic life with long lasting effects.

**13. DISPOSAL CONSIDERATIONS 13.1 Waste treatment methods**

**Product**

This combustible material may be burned in a chemical incinerator equipped with an afterburner and scrubber. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

---

**Contaminated packaging**

Dispose of as unused product.

**14. TRANSPORT INFORMATION**

**DOT (US)**

UN number: 2966 Class: 6.1 Proper shipping name: Thioglycol

Aldrich - M6250

Packing group: II

---

Page 7 of 9

Reportable Quantity (RQ):

Poison Inhalation Hazard: No

**IMDG**

UN number: 2966 Class: 6.1 Proper shipping name: THIOGLYCOL Marine pollutant:yes

**IAT A**

UN number: 2966 Class: 6.1 Proper shipping name: Thioglycol

**15. REGULATORY INFORMATION SARA 302 Components**

Packing group: II

Packing group: II

EMS-No: F-A, S-A

---

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

Fire Hazard, Acute Health Hazard, Chronic Health Hazard

**Massachusetts Right To Know Components**

2-Mercaptoethanol

**Pennsylvania Right To Know Components**

2-Mercaptoethanol

**New Jersey Right To Know Components**

2-Mercaptoethanol

**California Prop. 65 Components**

CAS-No. Revision Date 60-24-2 1993-04-24

CAS-No. Revision Date 60-24-2 1993-04-24

CAS-No. Revision Date 60-24-2 1993-04-24

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

**16. OTHER INFORMATION**

**Full text of H-Statements referred to under sections 2 and 3.**

Acute Tox. Aquatic Acute Aquatic Chronic Eye Dam. Flam. Liq.

H227

H301

H301 + H331 H310

H315

H317

H318

H331

H373

H400

**HMIS Rating**

Acute toxicity

Acute aquatic toxicity

Chronic aquatic toxicity

Serious eye damage

Flammable liquids

Combustible liquid.

Toxic if swallowed.

Toxic if swallowed or if inhaled

Fatal in contact with skin.

Causes skin irritation.

May cause an allergic skin reaction.

Causes serious eye damage.

Toxic if inhaled.

May cause damage to organs through prolonged or repeated exposure if swallowed. Very toxic to aquatic life.

Health hazard:

Chronic Health Hazard: \*

Aldrich - M6250



Flammability: 2 Physical Hazard 0

**NFPA Rating**

Health hazard: 3 Fire Hazard: 2 Reactivity Hazard: 0

**Further information**

Copyright 2015 Sigma-Aldrich Co. LLC. License granted to make unlimited paper copies for internal use only. The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See [www.sigma-aldrich.com](http://www.sigma-aldrich.com) and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

**Preparation Information**

Sigma-Aldrich Corporation Product Safety – Americas Region  
1-800-521-8956

Version: 5.5

Revision Date: 04/16/2015

Print Date: 07/09/2015



# 2-Mercaptoethanol

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

---

## 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
- 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 an dissolves the lipid membranes of the ce  
chemically alters the proteins and polysaccharides so that they don't  
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 display

---

## 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, 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



# F<sub>1</sub>

The first generation produced by a cross between parents that are homozygous for the trait. The F<sub>1</sub> 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 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 a hooded phenotype is masked. This results in the expression of a shaggy 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 ingredients to perform a PCR. The magnesium is needed for the enzyme (DNA polymerase) to function properly. The salt and buffer are needed for a stable 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 cut DNA.

---

## Related Glossary Terms

Drag related terms here



Seed-coat color



# Short awn



---

## Related Glossary Terms

Drag related terms here

---

**Index**

Find Term

Chapter 1 - Introduction



# Sickle Cell

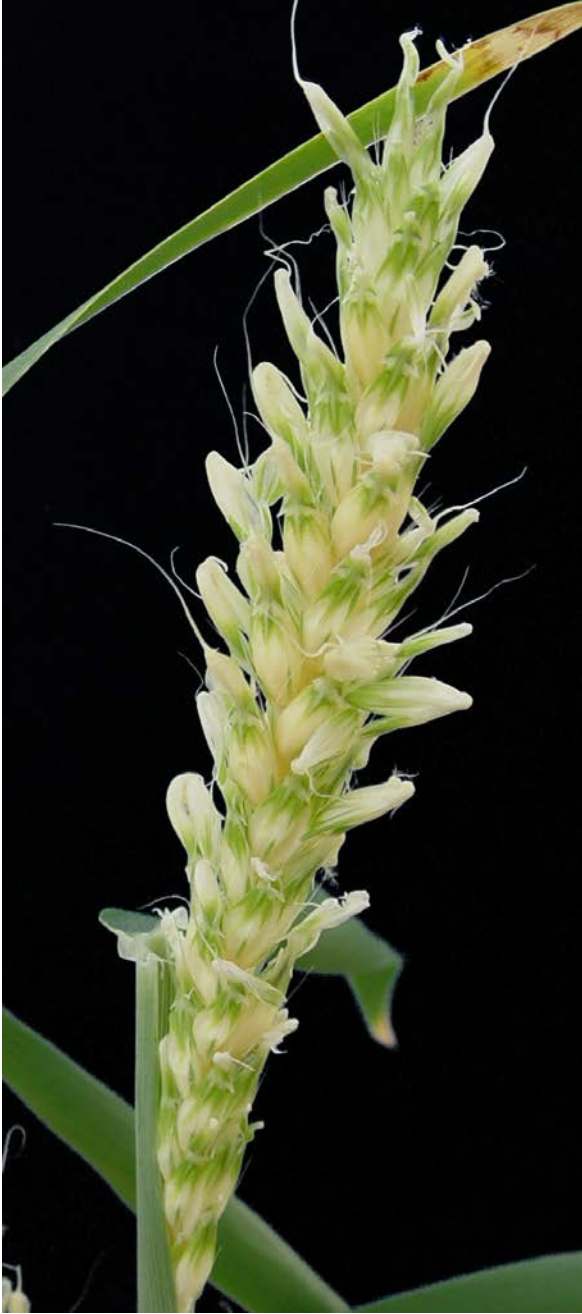
Hereditary blood disorder, characterized by red blood cells that are an rigid, sickle shape. This decreases the cells' flexibility and can cause complications.

---

## 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

---

**Index**

Find Term

Chapter 1 - Introduction

# 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

---

## Index

Find Term

### Chapter 2 - Polymerase Chain Reaction of the Kap Gene

Chapter 2 - Polymerase Chain Reaction of the Kap Gene

Chapter 2 - Polymerase Chain Reaction of the Kap Gene

Chapter 2 - Polymerase Chain Reaction of the Kap Gene

Chapter 3 - Polymerase Chain Reaction of the Vrs1 Gene

Chapter 3 - Polymerase Chain Reaction of the Vrs1 Gene

Chapter 3 - Polymerase Chain Reaction of the Vrs1 Gene

Chapter 3 - Polymerase Chain Reaction of the Vrs1 Gene

# Tay Sachs

A recessive genetic disorder that causes progressive deterioration of m that begins around six months of age and usually results in death by a

---

## Related Glossary Terms

Drag related terms here



# TBE Buffer

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

---

## 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  
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