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THE **BIOTECHNOLOGY EDUCATION** COMPANY®

Includes EDVOTEK's All-NEW
EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes:
2640, 1400, 1100, 700, 600, 400, 200

Edvo-Kit #
336

Edvo-Kit #336

Determining Quick Plant™ Genetics Using PCR

Experiment Objective:

The object of this experiment is to introduce students to the concept of genetic linkage by using the polymerase chain reaction to amplify DNA from wild-type and mutant *Arabidopsis* plants.

See page 3 for storage instructions.

NOTE:
PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.

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Table of Contents

Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

	Component	Storage	Check (✓)
A	PCR EdvoBeads™ Each PCR EdvoBead™ contains • dNTP Mixture • *Taq* DNA Polymerase Buffer • *Taq* DNA Polymerase • $MgCl_2$ • Reaction Buffer	Room Temperature	❑
B	Primer mix concentrate	-20°C Freezer	❑
C	EdvoQuick™ DNA ladder	-20°C Freezer	❑
D	Control DNA concentrate	-20°C Freezer	❑
E	TE buffer	-20°C Freezer	❑
F	Proteinase K	Room temp., desiccated	❑
G	NaCl	Room temp.	❑
H	DNA Extraction Buffer	Room temp.	❑

This kit contains enough reagents for 10 lab groups.

Sample volumes are very small. It is important to quick spin the tube contents in a microcentrifuge to obtain sufficient volume for pipetting. Spin samples for 10-20 seconds at maximum speed.

NOTE: ***Components B and D are supplied in a concentrated form and require dilution prior to setting up PCR reactions.***

Reagents & Supplies

Store all components below at room temperature.

Component	Check (✓)
• Quick Plant™ *Arabidopsis* seeds (Wild-type and *glabra*)	❑
• Peat pods for plant growth	❑
• Plant Homogenization pestles with tubes	❑
• UltraSpec-Agarose™	❑
• 50x Electrophoresis Buffer	❑
• 10x Gel Loading Solution	❑
• InstaStain® Ethidium Bromide	❑
• FlashBlue™ liquid stain	❑
• Snap-top microcentrifuge tubes (1.5 ml)	❑
• PCR tubes (0.2 ml)	❑
• Wax beads (for thermal cyclers without heated lid)	❑

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Requirements *(NOT included in this experiment)*

- Thermal cycler (EDVOTEK Cat. # 541 highly recommended) or three waterbaths*
- Waterbath for 56° C incubation (EDVOTEK® Cat. #539 highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge (10,000Xg)
- Plant lights (recommended)
- UV Transilluminator or UV Photodocumentation system (use if staining with InstaStain™ Ethidium Bromide)
- UV safety goggles
- White Light visualization system (optional - use if staining with FlashBlue™)
- Automatic micropipets (5-50 µl, 100-1000 µl) with tips
- Microwave or hot plate
- 250 ml flasks or beakers
- Hot gloves
- Disposable laboratory gloves
- Ice buckets and ice
- Distilled or deionized water
- Isopropanol
- Ethanol

*If you do not have a thermal cycler, PCR experiments can be conducted, with proper care, using three waterbaths. However, a thermal cycler assures a significantly higher rate of success.

Background Information

Arabidopsis thaliana is a small, weed-like plant from the mustard family, Brassicaceae (Cruciferae). In spite of its humble appearance, *Arabidopsis* has become a superstar for plant geneticists and molecular biologists. There are several reasons for its success. First, the small size of the plants allows for large numbers to be grown in a small space in the laboratory, growth chamber or greenhouse. Second, *Arabidopsis* has a very short life cycle. Plants from seeds planted today will begin flowering in only three to four weeks. This is an advantage for geneticists because they can make experimental crosses and raise many generations in a very short period of time. Third, *Arabidopsis* has a very small genome consisting of 5 chromosomes. The amount of DNA normally found in *Arabidopsis* cells is small compared to that of other plants. Some plant species are known to contain 10,000 times as much DNA per cell as *Arabidopsis*. The small size of the *Arabidopsis* genome has made it possible to determine its entire nucleotide sequence. This task was completed in 2000. Annotating and identifying the genes in the sequence and assigning functions to them, will probably take many more years.

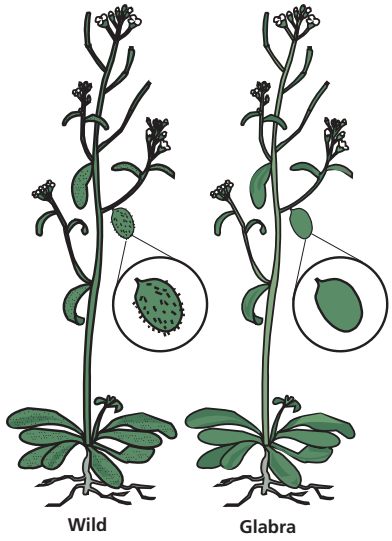


Figure 1: Wild and mutant *glabra* strains. The *gl1-1 glabra* are hairless mutants that lack fine glandular hairs (trichomes).

The same features of *Arabidopsis* that make it an attractive organism for research also make it useful in the classroom. The plant can be grown in large numbers and in a small space under classroom conditions. Genetics experiments can be completed in a single semester. Large numbers of interesting mutants have been identified and characterized, and several have been selected as especially useful for education.

Examples of mutant characteristics are described below:

- *gai1* is a gibberellic acid insensitive dwarf. This *Arabidopsis* plant is much smaller than the wild-type.
- *ap1-1* and *ap3-3*, are homeotic mutants. Homeotic mutations have the effect of converting one organ or body part into another; ap stands for apetala. The name refers to the phenotype of the mutants, lacking petals because they have been converted into other flower parts. Note that although both of these mutations produce similar phenotypes, they are defects in different genetic loci as indicated by their numbering.
- *fus3-3 , fusca,* is a mutant in which the germinating seeds are splotched with reddish brown color. Normally, seedlings are expected to be a uniform light green color.
- *var* mutants are variegated. Leaves are splashed with patches of white.
- *yi1* mutants have a yellow inflorescence. The flower buds of this mutant are a very pale green – yellowish color and the flower petals are off-white.
- *tt2-1* mutants have a transparent testa (or uncolored seed coat). Normally, seed coats are brown and this makes the seeds brown. Transparent testa mutants, therefore, produce yellow, rather than brown seeds. Since the seed coat has no color, the seeds show the color of the embryo inside.
- The *gl1-1 glabra* are hairless mutants that are selected for inclusion in this mapping experiment. This mutant lacks the fine glandular hairs (trichomes) normally found covering the surface of an *Arabidopsis* leaf.

MAPPING STRATEGY

There are many advantages of genetic mapping vs. classical plant breeding. With classical plant breeding/genetics, many crosses are required and many f1 lines must be maintained to reach a final result. With genetic mapping, an assay from the DNA of a single cross will yield many DNA polymorphic markers.

Traditionally, genes have been located, or mapped to specific loci on chromosomes by the technique of recombination mapping. This technique takes advantage of the fact that genes located very close together on a chromosome are often inherited together as a package. The closer two genes are to one another, the less likely they are to be separated by recombination. So, a gene is mapped by measuring the frequency of recombination between the gene of interest and other genes that have already been placed on the chromosome.

This strategy for mapping genes is limited however, by the number of genes that have already been mapped. Producing a very detailed map by recombination analysis requires many genes. Molecular biology has extended our ability to map genes by providing convenient genetic markers in numbers that literally saturate the chromosomes. Using molecular markers rather than Mendelian traits as chromosomal landmarks for mapping means that genes can be placed very precisely on the genetic map.

DNA EXTRACTION

Every method for extraction of DNA includes some common features: tissues are disrupted to release DNA, cellular debris is removed, and DNA is precipitated to separate it from other cellular components. The method outlined in this experiment includes each of these steps. First, small amounts of plant leaf tissue from *Arabidopsis* plants carrying the gene to be mapped and from mutant *Arabidopsis* plants are ground into a fine suspension in extraction buffer. This buffer contains a chelating agent (EDTA) to protect DNA from the activity of nucleases released from the tissue as the cells are disrupted. It also includes salt and a detergent (SDS) which will disrupt cellular membranes. Second, the plant tissue is incubated in a hot water bath to facilitate cell lysis. Cell debris is removed from the preparation by centrifugation and the pelleted material is ground a second time to maximize DNA yield. After centrifuging a second time, DNA is precipitated from the clarified supernatant with isopropanol. The DNA prepared by this method is sufficiently purified to work as a template in the polymerase chain reaction step that follows.

POLYMERASE CHAIN REACTION

Since its discovery in the mid 1980s, the polymerase chain reaction (PCR) has revolutionized biological science. The enormous utility of PCR is based on its ease of use and its ability to amplify DNA. PCR amplification uses an enzyme known as *Taq* DNA polymerase. This enzyme, originally purified from a bacterium that inhabits hot springs, is stable at very high (near boiling) temperatures. Also included in the PCR reaction mixture are short (15-30 nucleotide) synthetic oligonucleotides, known as primers and the extracted DNA that contains the region to be amplified, known as the "target".

In the first step of the PCR reaction (Figure 2), known as denaturation, the target complementary DNA strands are melted (separated) from each other at 94°C, while the *Taq* DNA polymerase remains stable. In the second step, known as annealing, the sample is cooled to an intermediate temperature (usually between 37°C and 65°C) to allow hybridization of the two primers to the two strands of the target DNA. In the third PCR step (Figure 2), known as extension, the temperature is raised to 72°C. At this temperature, the *Taq* DNA polymerase is maximally active and adds nucleotides to the primers to complete the synthesis of the new complementary strands to the target region. These three steps - denaturation, annealing, and extension– constitute one PCR "cycle". This process is typically repeated for 20-40 cycles, amplifying the target sequence exponentially. PCR is performed in a thermal cycler that is programmed to heat, cool and maintain samples at precise temperatures for varying time intervals.

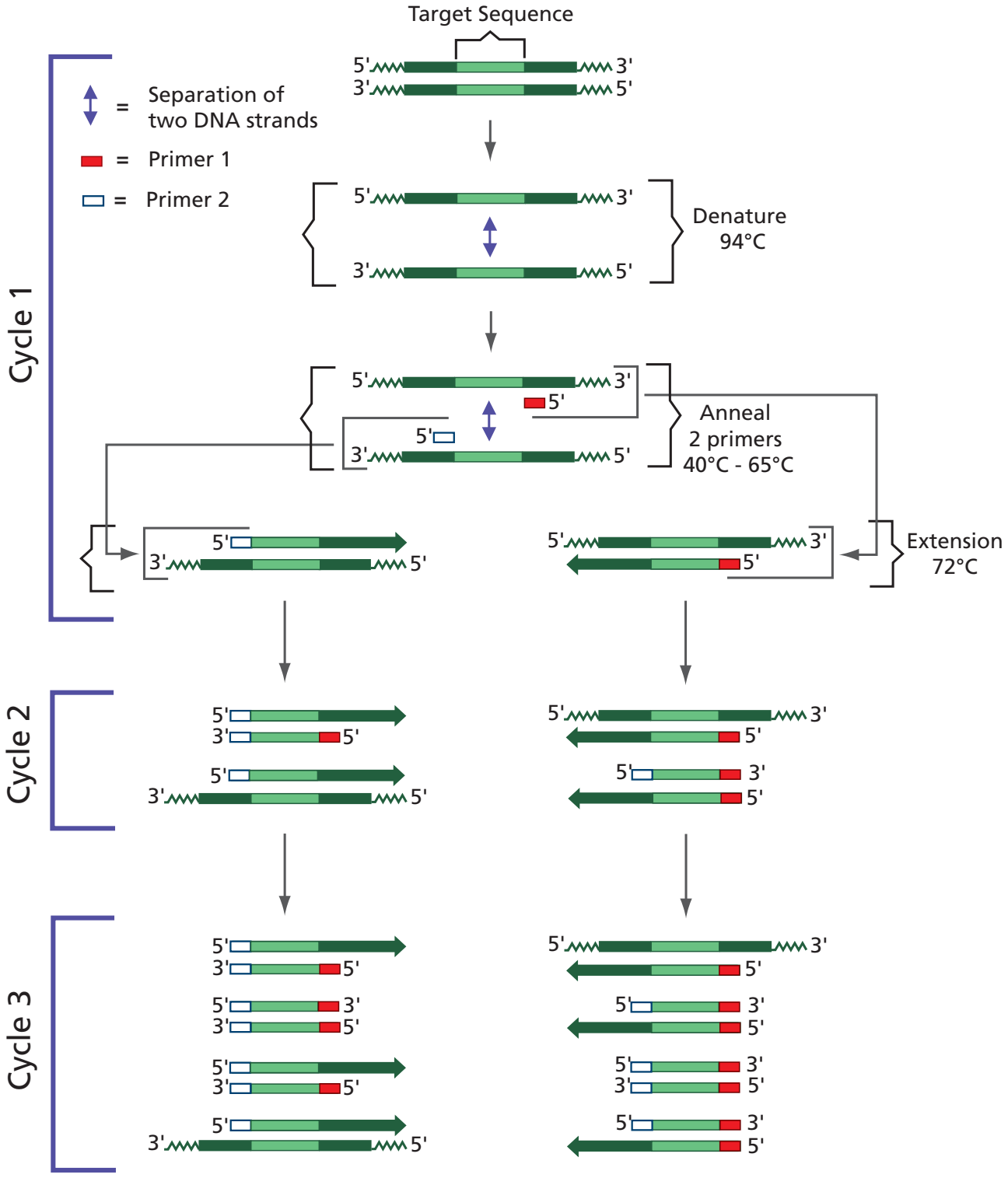


Figure 2: DNA Amplification by the Polymerase Chain Reaction

Experiment Overview

EXPERIMENT OBJECTIVE

The object of this experiment is to introduce students to the concept of genetic linkage by using the polymerase chain reaction to amplify DNA from wild-type and mutant *Arabidopsis* plants.


Wear gloves and safety goggles

BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, the extracted *Arabidopsis* (*glabra* and wild-type) DNA will be amplified at two separate target sequences on chromosomes 1 and 3. The amplified region (519 base pairs) on chromosome 3 is linked to the *glabra* gene, while the target on chromosome 1 (1481 base pairs) is unlinked. Comparison of the wild-type and *glabra* PCR products experimentally demonstrate the concept of genetic linkage

IMPORTANT

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.

MODULE I: 2-4 weeks
Growing QuickPlants™ *Arabidobsis thaliana*

↓

MODULE II: 3.5 hours
Isolation of Genomic DNA from *Arabidobsis*

↓

MODULE III: 2 hours
PCR of Genomic DNA from *Arabidobsis*

↓

MODULE IV: 50-70 min.
Agarose Gel Electrophoresis

↓

MODULE V: 5-30 min.
Staining Agarose Gels

LABORATORY NOTEBOOKS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:
- Record (draw) your observations, or photograph the results.

Following the Experiment:
- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Module I: Growing Quick Plants™ - *Arabidopsis thaliana*

1. Hydrate peat pods in warm water for 30 minutes. Drain off any excessive water and place the prepared pods in a shallow plastic tray (e.g. a plastic food container or a small tray). Several peat pods can be put together in a small container (Figure 3).
2. Add 0.5 ml water to each tube of seeds. Use a small transfer pipet to spread the seeds evenly on the soil surface. Each genotype should be distributed between five pods. Be sure to keep track of the genotypes of the seeds in each pod.
3. Place the seeds directly under fluorescent lights or in direct sunlight from a window. Do not cover the seeds with soil or a lid; the seeds require light for germination.
4. Keep pods/seeds moist and place lights very close to pods while the seeds germinate. This will take approximately 4-7 days.
5. After the seeds germinate, be sure that the peat pods remain moist – the plants will not thrive if the soil mixture is too dry or too wet. For optimal results, mist the plants daily with a dilute (1/4 strength) solution of balanced commercial fertilizer.
6. Allow the plants to grow for 2-4 weeks before performing the experiment. For best results, the seedlings should be approximately 4-6 cm.

HELPFUL HINTS AND NOTES

Planting the Seeds:

- To help break seed dormancy, refrigerate the seeds for 2-3 days before planting. This "tricks" the seeds into signaling that the cold season is over and that the growing season can begin.
- If not using the included peat pods, students can use a peat-based potting mix. Use light soil mixtures with ample peat moss, and sterilize before planting in order to avoid any pest contamination. Alternatively, use commercially prepared mixes, such as Metromix 350 or ProMix BX. The surface of the soil should be approximately 1 cm from the top of the pot. Be sure that the seeds are not covered by the soil because light is required for germination.
- If your classroom is dry, cover the samples with clear plastic wrap to maintain the appropriate humidity for germination. Be sure to perforate the plastic wrap to allow for airflow. Remove the plastic wrap after sprouting is observed.

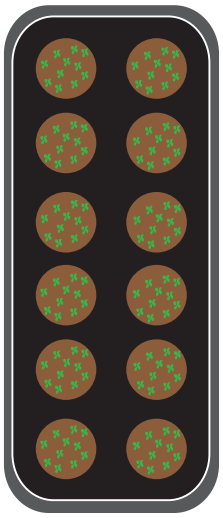


Figure 3:
Seedlings in their pods.

Temperature:

- The optimal temperature range for germination and growth of Quick Plants™ is 22- 25°C.
- Before beginning the experiment, be sure your classroom maintains a constant temperature at night and over the weekend. Temperatures above 28°C and below 18°C will affect growth and development.

Lighting:

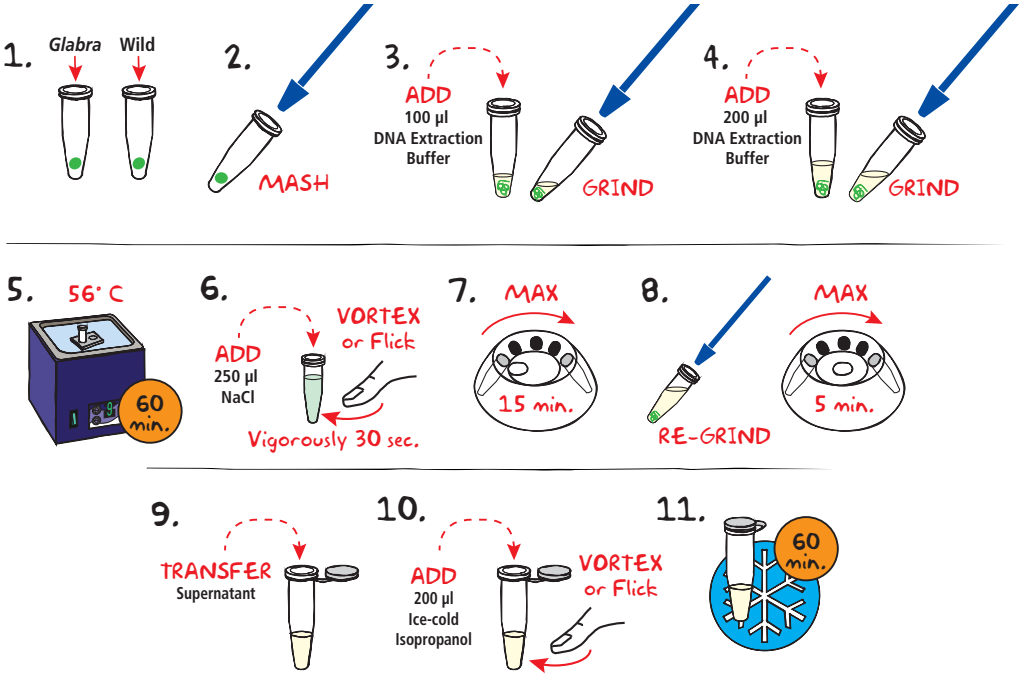
More than any other factor, light determines how quickly the plants will grow and develop. With proper lighting, germination should occur within seven days. Plants may take another 2-4 weeks to grow to the proper size.

- Fastest growth is under continuous fluorescent light (no day-night cycle is necessary). We recommend using workshop clamp lights, which are easily and inexpensively configured in a classroom or lab. See Appendix D for directions on creating a plant growth chamber. This lighting condition may produce compact sized plants.
- On a bright windowsill, the plants may take 1-2 weeks longer to develop, but are larger in size.
- Slowest growth occurs under low light conditions, such as a poorly lit windowsill.

Watering:

- Quick Plants™ are amazingly hardy after germination. Water plants as needed to avoid water stress and to prevent fungal growth on the soil surface.
- If contamination does appear, allow the surface of the pods to dry and scrape the fungus from the soil surface with care.

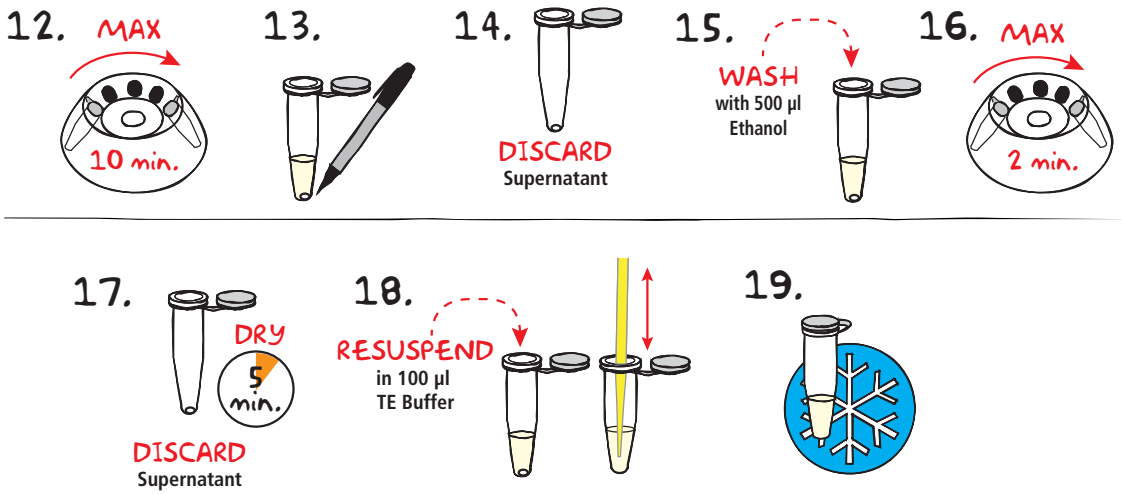
Module II: Isolation of Genomic DNA from *Arabidopsis*



1. **HARVEST** 4-6 seedlings (~1.5 cm tall, 1-3 weeks old), or 5-6 leaves from a mature plant (4-5 mm in diameter) into a labeled microcentrifuge tube with pestle. Label should include group name/number and the plant genotype. Place *glabra* mutant seedlings into one tube and wild-type seedlings into another. Keeps tubes and pestles separate throughout the entire experiment to avoid cross contamination.
2. Use the pestle to partially **MASH** the tissue for 10 seconds. Use a separate pestle for each tube.
3. **ADD** 100 µl of DNA Extraction Buffer to each tube and continue grinding the tissue for 10 seconds.
4. **ADD** an additional 200 µl of DNA Extraction Buffer to each tube and grind the tissue again for 10 seconds.
5. **INCUBATE** the tubes in a waterbath at 56° C for one hour.
6. After the 56° C incubation, **ADD** 250 µl NaCl solution to each tube and mix well by vortexing or flicking for 30 seconds.
7. **CENTRIFUGE** the tubes at full speed (10,000xg) for 15 min.
8. Using the appropriate pestle, **RE-GRIND** the pelleted material in each tube. **CENTRIFUGE** the sample at full speed (10,000xg) for 5 min.
9. Carefully **TRANSFER** the supernatant from each tube into a fresh, labeled microcentrifuge tube being careful not to disturb the pellet. Discard the tubes with pellets.
10. **PRECIPITATE** the DNA from the supernatant by adding 200 µl of ice-cold 100% isopropanol. **MIX** well by vortexing or flicking the tube.
11. **INCUBATE** the tubes in the freezer for at least one hour. For convenience, samples can be left in the freezer overnight.

OPTIONAL STOPPING POINT:
Store the samples at -20° C if they will not be used immediately.

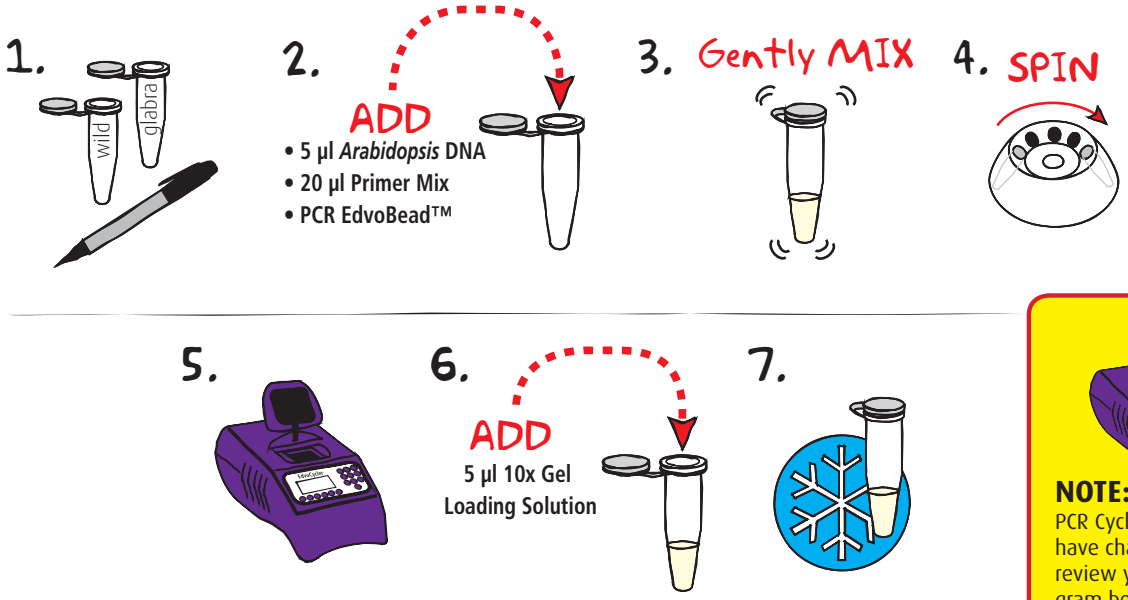
Module II: Isolation of Genomic DNA from *Arabidopsis*, continued



12. **INSERT** the tubes in the rotor so that the hinges are facing towards the outside edge. **CENTRIFUGE** the sample for 10 minutes at full speed (10,000xg). The precipitated plasmid DNA will form a small, white pellet at the bottom of the hinge side of tube after centrifugation.
13. After centrifugation, a very small DNA pellet should be visible at the bottom of the tube. With a marker, **CIRCLE** the location of the DNA pellet.
14. Carefully **REMOVE** and **DISCARD** all the supernatant, leaving the pelleted DNA at the bottom of the tube. Take care to avoid the pellet when removing the supernatant, as it may come loose.
15. **WASH** the pellet with 500 µl 70% Ethanol.
16. **CENTRIFUGE** the sample at full speed (10,000Xg) for 2 minutes.
17. Carefully **REMOVE** and **DISCARD** the supernatant and allow the DNA pellet to dry for 5 minutes.
18. Completely **RESUSPEND** the pellet in 100 µl of TE by pipetting up and down several times or by vortexing vigorously.
19. **PLACE** tubes in ice. **PROCEED** to Module III: PCR of Genomic DNA from *Arabidopsis*.

OPTIONAL STOPPING POINT:
Store the DNA at -20° C if it will not be used immediately.

Module III: PCR of Genomic DNA from *Arabidopsis*



NOTE:
PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.

Perform one PCR reaction for <u>each</u> plant type.

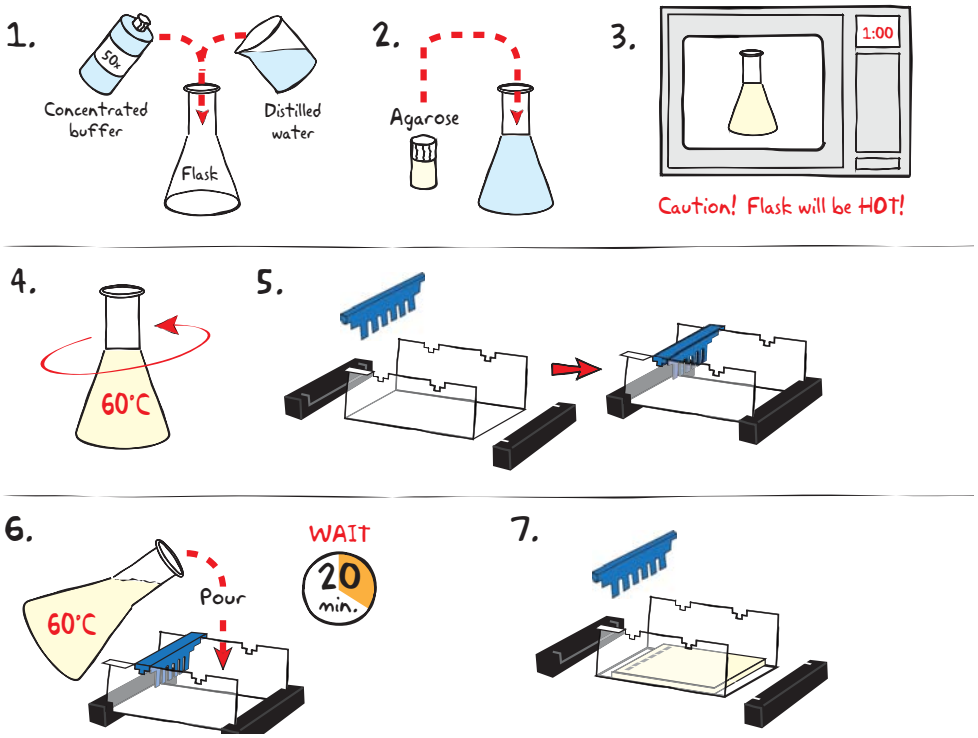
1. **LABEL** each PCR tube with the appropriate name ("wild" or "*glabra*") and group name/number.
2. **ADD** 5 µl extracted *Arabidopsis* DNA (wild or *glabra*), 20 µl Primer mix, and one PCR EdvoBead™ to a labeled 0.2 ml PCR tube.
3. **MIX** the PCR sample. Make sure the PCR EdvoBead™ is completely dissolved.
4. **CENTRIFUGE** the sample for a few seconds to collect the liquid at the bottom of the tube.
5. **AMPLIFY** DNA using PCR.
   <u>PCR cycling conditions:</u>
   Initial denaturation 94°C for 5 minutes
   94° C for 30 seconds
   54° C for 30 seconds } 35 cycles
   72° C for 120 seconds
   Final Extension 72° C for 4 minutes
6. After the completion of the cycling, **ADD** 5 µl of 10x Gel Loading Solution to each tube.
7. **PLACE** tubes on ice. **PROCEED** to Module IV: Agarose Gel Electrophoresis.

NOTES AND REMINDERS:
If your thermal cycler does not have a heated lid, it is necessary to overlay the PCR reaction with wax to prevent evaporation. See Appendix B for guidelines.

This kit includes enough DNA for 5 control reactions. At least one control reaction should be performed per class to confirm that PCR was successful.

OPTIONAL STOPPING POINT:
The PCR samples may be stored at -20° C for electrophoresis at a later time.

Module IV: Agarose Gel Electrophoresis



IMPORTANT:

7 x 14 cm gels are recommended. Each gel can be shared by 2 groups. Place well-former template (comb) in the first set of notches.

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

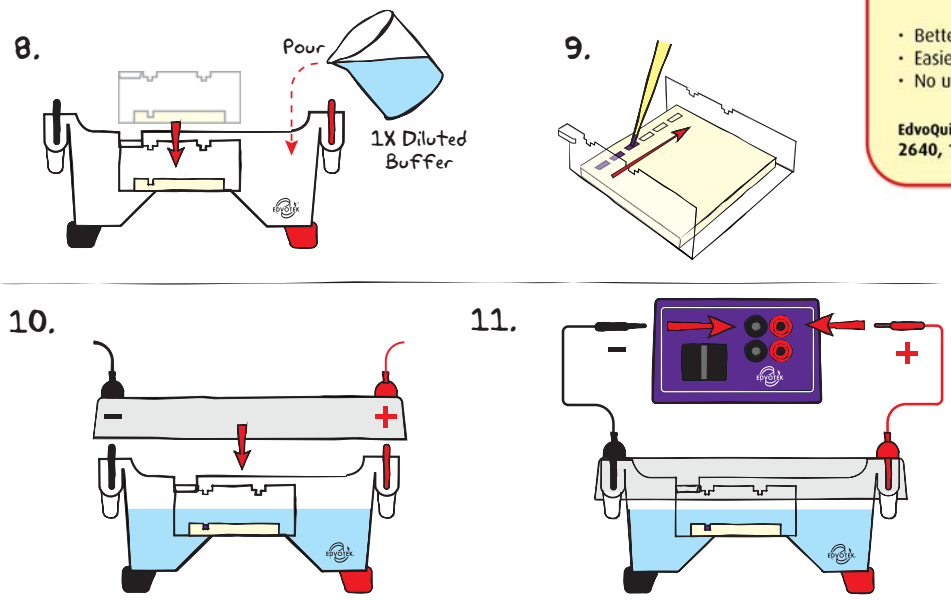
Wear gloves and safety goggles

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 1.0% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.5 ml	24.5 ml	0.25g	25 ml
7 x 14 cm	1.0 ml	49.0 ml	0.50 g	50 ml

Module IV: Agarose Gel Electrophoresis



Includes EDVOTEK's All-NEW EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes:
2640, 1400, 1100, 700, 600, 400, 200

Wear gloves and safety goggles

Reminder:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** 25 µl into the well in the order indicated by Table 1.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). The tracking dye should migrate 6 cm on a 7x14 cm gel.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING** the agarose gel.

Table 1

Lane	Recommended
1	EdvoQuick™ DNA Ladder
2	Control DNA
3	Wild type PCR - Group 1
4	*Glabra* PCR - Group 1
5	Wild type PCR - Group 2
6	*Glabra* PCR - Group 2

Table B — 1x Electrophoresis Buffer (Chamber Buffer)

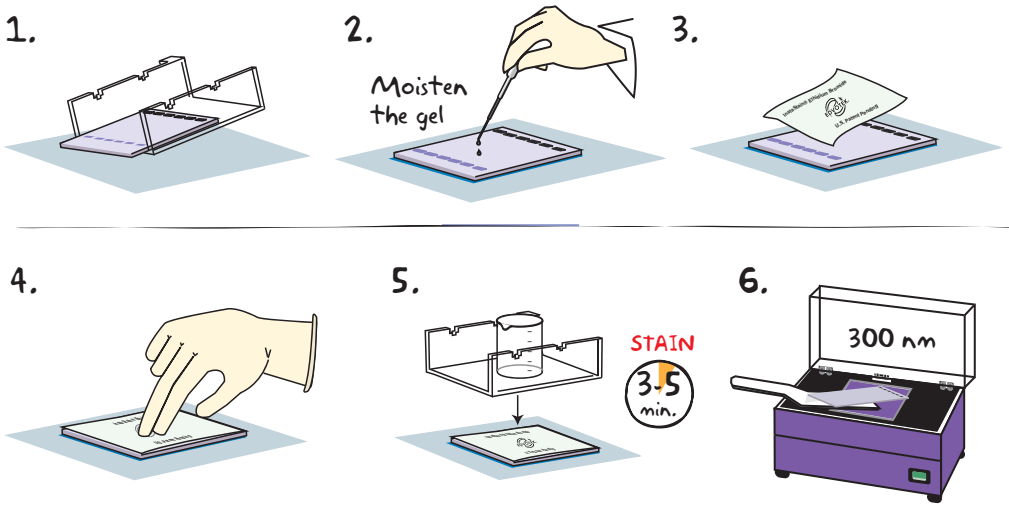
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 ml
M12	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

Table C — Time and Voltage Guidelines (1.0% - 7 x 14 cm Agarose Gel)

Volts	Recommended Time: Minimum	Maximum
125	55 min.	1 hour 15 min.
70	2 hours 15 min.	3 hours
50	3 hours 25 min.	5 hours

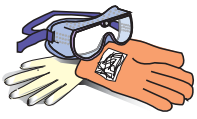
Module V-A: Staining with InstaStain® Ethidium Bromide

Preferred Method



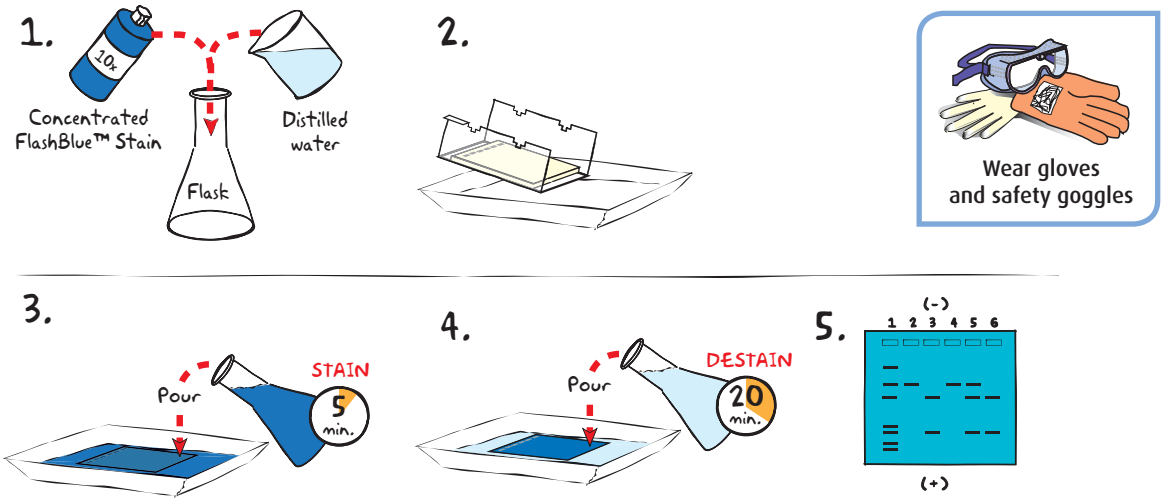
1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface. **DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.**
2. **MOISTEN** the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, **REMOVE** and **DISCARD** the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). **PLACE** the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need 1 card to stain a 7 x 7 cm gel and 2 cards to stain a 7 x 14 cm gel.
4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. **STAIN** the gel for 3-5 minutes.
6. **REMOVE** the InstaStain® Ethidium Bromide card(s). **VISUALIZE** the gel using a mid-range ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!



Wear gloves and UV safety goggles

Module V-B: Staining with FlashBlue™



1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
5. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Alternate Protocol:

1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH_2O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
4. Carefully **REMOVE** the gel from the staining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Study Questions

1. Describe the methods involved in amplification of plant DNA from start to finish.
2. What can interfere with obtaining successful PCR results?
3. What are the advantages of using a genetic mapping strategy vs. traditional plant breeding/crossing?
4. How can mapping a plant such as *Arabidopsis* help with other plant species or in other areas of plant breeding and genetics?

Instructor's Guide

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Prior to starting this experiment, carefully check the list of Components and Requirements on pages 3 and 4 to ensure that you have all the necessary components and equipment.

APPROXIMATE TIME REQUIREMENTS

MODULE	PRELAB	EXPERIMENT
I	20 min.	2-4 weeks
II	45 min.	3.5 hours
III	45 min.	2 hours
IV	60 min.	1-1.5 hours
V	10 min.	30 min.

Some modules of this experiment may require multiple laboratory periods. The experiment can be temporarily stopped after the completion of each module or at designated stopping points. Student results will not be compromised if the experiment is paused at these times. Please consider this in the planning and the implementation of this experiment.

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION

This section outlines the recommended prelab preparations and approximate time requirements to complete each prelab activity.

Preparation For:	What to do:	When:	Time Required:
Module I: Growing QuickPlants™ *Arabidopsis thaliana*	Check classroom temperature overnight/ on weekend	Before growing the plants.	2-3 days
Module II: Isolation of Genomic DNA from *Arabidopsis*	Prepare lysis buffer	No more than one hour before performing the experiment.	10 min.
	Prepare and aliquot reagents	Up to one day before performing the experiment.	30 min.
	Equilibrate waterbaths.	One hour before performing the experiment.	5 min.
Module III: PCR of Genomic DNA from *Arabidopsis*	Prepare and aliquot reagents	One day to 30 min. before performing the experiment.	30 min.
	Program Thermal Cycler	One day to 30 min. before performing the experiment.	15 min.
Module IV: Agarose Gel Electrophoresis	Prepare diluted electrophoresis buffer	Up to one day before performing the experiment.	45 min.
	Prepare molten agarose and pour gel		
Module V: Staining Agarose Gels	Prepare staining components	The class period or overnight after the class period.	10 min.

Pre-Lab Preparations

MODULE I: GROWING QUICK PLANTS™

Plan to have plants ready for harvest on the day of the lab. Allow 3-4 weeks for adequate growth. See Growing Quick Plants™ in the Experiment Procedures section.

Before beginning the experiment, be sure your classroom maintains a constant temperature at night and over the weekend. Temperatures above 28°C and below 18°C will affect growth and development.

FOR MODULE I
Each Group should receive:

- Quick Plant™ Seeds (*glabra* and wild-type)
- Peat Pods or Soil

MODULE II: ISOLATION OF GENOMIC DNA FROM *Arabidopsis*

This module takes three and a half hours. If you cannot perform this entire module in one class period, the experiment can be stopped following Step 11. Samples can be stored in the freezer (-20° C) for up to one week. The first eleven steps will take approximately two hours and the last eight steps will take thirty minutes.

FOR MODULE II
Each group should receive:

- *Arabidopsis glabra* plants
- *Arabidopsis* wild-type plants
- 2 microcentrifuge tubes with pestle
- 1 ml DNA Extraction Buffer (H)
- Ice-cold 100% isopropanol
- 70% Ethanol
- 1 ml TE buffer
- 1 ml NaCl solution
- Additional microcentrifuge tubes

Day of the Lab:

1. Set filled waterbath to 56° C.
2. If a precipitate has formed in the DNA extraction buffer, warm at 37° C to redissolve.
3. Prepare DNA Extraction Buffer:

   NOTE: Prepare no more than one hour before starting the experiment.

   - Add 200 µl of DNA Extraction Buffer (H) to each tube of Proteinase K and allow the pellets to hydrate for a couple of minutes.
   - Add the dissolved Proteinase K back to the 10 ml of DNA Extraction Buffer and mix.
   - Aliquot 1 ml for each group and keep on ice.
4. Aliquot 1 ml of NaCl solution (G) for each group.
5. Aliquot 1 ml of TE Buffer (E) for each group.
6. Place bottle of 100% isopropanol on ice.
7. Prepare 70% ethanol.

Pre-Lab Preparations

MODULE III: PCR OF GENOMIC DNA FROM *Arabidopsis*

FOR MODULE III
Each group should receive:

- Two PCR tubes with PCR EdvoBeads™
- 50 µl Primer Mix
- 20 µl Gel Loading Solution
- 6 µl Control DNA (optional)
- Extra PCR reagents (optional)

Preparation of the Primer Mix:

1. Thaw the Primer Mix Concentrate (B) on ice.
2. Add 1 ml of TE Buffer (E) to the tube of Primer Mix Concentrate. Cap tube and mix.
3. Label 13 microcentrifuge tubes "Primer". Aliquot 50 µl of the diluted Primer Mix into the 10 microcentrifuge tubes. Place the tubes on ice until they are needed.
4. Distribute one tube of diluted Primer to each student pair.

Preparation of the Control DNA:

This kit includes enough DNA for 5 control samples. The DNA was prepared from *glabra* plants.

1. Thaw the tube of Control DNA Concentrate (F) on ice.
2. Add 20 µl of TE Buffer (E) to the tube containing Control DNA Concentrate. Pipet up and down to mix.
3. Aliquot 6 µl of the diluted DNA control into the appropriate microcentrifuge tubes. Place the tubes on ice until they are needed.
4. Distribute tubes of diluted DNA. Each control will be shared by two groups.

Additional Materials:

- Dispense 20 µl of 10X Gel Loading Solution per tube. Label these 10 tubes "10x Solution". Distribute one tube per student group.
- Each group will also receive two PCR tubes and two PCR EdvoBeads™.
- Groups receiving the control DNA should receive an extra PCR tube and PCR EdvoBead™. Alternatively, the instructor can prepare the additional control samples and distribute to students after PCR.

Programming the Thermal Cycler:

The Thermal cycler should be programmed as outlined in Module III in the Student's Experimental Procedure.

- Accurate temperatures and cycle times are critical. A pre-run for one cycle (takes approximately 3 to 5 min.) is recommended to check that the thermal cycler is properly programmed.
- For thermal cyclers that do not have a heated lid, it is necessary to place a layer of wax above the PCR reactions in the microcentrifuge tubes to prevent evaporation. See Appendix B for instructions.

Pre-Lab Preparations

MODULE IV: AGAROSE GEL ELECTROPHORESIS

Preparation of Agarose Gels

This experiment requires one 1.0% agarose gel per two student groups. A 7 x 14 cm gel is recommended. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation:
Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module III in the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

Batch Gel Preparation:
To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix C.

Preparing Gels in Advance:
Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20° C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

Additional Materials:
Each 1.0% gel should be loaded with the EdvoQuick™ DNA ladder (C) and PCR reactions from two student groups.

- Aliquot 26 µl of the EdvoQuick™ DNA ladder (C) into labeled microcentrifuge tubes and distribute one tube of EdvoQuick™ DNA ladder per gel.

NOTE:
Accurate pipetting is critical for maximizing successful experiment results. This experiment is designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipets, we recommended performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this advanced level experiment.

FOR MODULE IV
Each Group should receive:
- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™ Powder
- EdvoQuick DNA ladder (C) (26 µl)

NOTE:
QuickGuide instructions and guidelines for casting various agarose gels can be found our website. **www.edvotek.com/quick-guides**

Pre-Lab Preparations

MODULE V: STAINING

InstaStain® Ethidium Bromide *(PREFERRED METHOD)*

InstaStain® Ethidium Bromide provides the sensitivity of ethidium bromide while minimizing the volume of liquid waste generated by staining and destaining a gel. An agarose gel stained with InstaStain® Ethidium Bromide is ready for visualization in as little as 3 minutes! Each InstaStain® card will stain 49 cm^2 of gel (7 x 7 cm). You will need 2 cards to stain a 7 x 14 cm gel.

FOR MODULE V-A
Each Group should receive:
- 2 InstaStain® cards per 7 x 14 cm gel

Wear gloves and safety goggles

Use a mid-range ultraviolet transilluminator (Cat. #558) to visualize gels stained with InstaStain® Ethidium Bromide. **BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!**

- Standard DNA markers should be visible after staining even if other DNA samples are faint or absent. If bands appear faint, repeat staining with a fresh InstaStain card for an additional 3-5 min. If markers are not visible, troubleshoot for problems with electrophoretic separation.
- Ethidium bromide is a listed mutagen. Wear gloves and protective eyewear when using this product. UV protective eyewear is required for visualization with a UV transilluminator.
- InstaStain® Ethidium Bromide cards and stained gels should be discarded using institutional guidelines for solid chemical waste.

FlashBlue™

FlashBlue™ can be used as an alternative to Ethidium Bromide in this experiment. However, FlashBlue™ is less sensitive than InstaStain® Ethidium Bromide and will take a longer time to obtain results.

FOR MODULE V-B
Each Group should receive:
- 10 ml 10X concentrated FlashBlue OR 100 ml 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

FlashBlue™ stain, however, is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to "equilibrate" in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

Photodocumentation of DNA (Optional)

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

Please refer to the kit insert for the Answers to Study Questions

Appendices

A EDVOTEK® Troubleshooting Guide

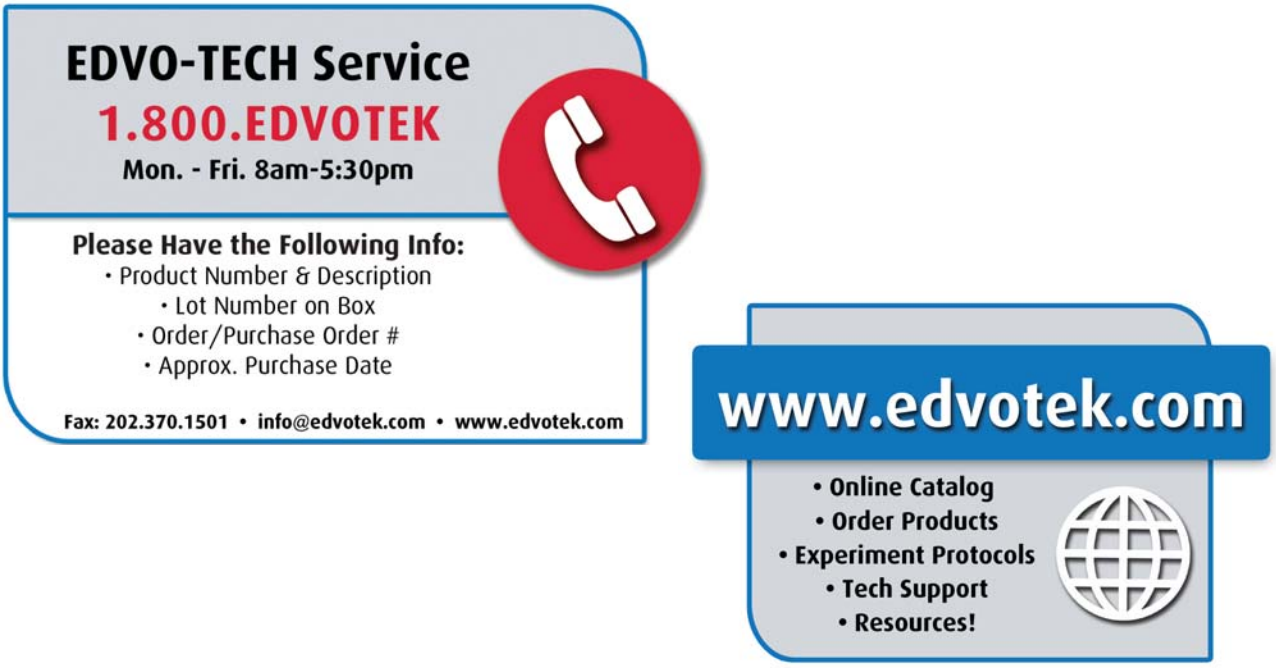
B Preparation and Handling of PCR Samples With Wax

C Bulk Preparation of Agarose Gels

D Creating a Plant Growth Chamber

Safety Data Sheets:

Now available for your convenient download on **www.edvotek.com/Safety-Data-Sheets**



Appendix A
EDVOTEK® Troubleshooting Guides

PLANT DNA EXTRACTION

PROBLEM:	CAUSE:	ANSWER:
Poor DNA Extraction	Samples not mixed well enough during extraction	In addition to flicking the tube, vortex or pipet up and down to mix the sample.
	Proteinase K inactive because it was prepared too far in advance.	Prepare Proteinase K within one hour of use.
	Water baths not at proper temperature	Use a thermometer to confirm water bath set point.
	Not enough DNA	Extracting DNA from plants can be challenging.
	Not enough plant tissue	Use more tissue (4-6 seedlings or 5-10 leaves from a mature plant.
	No DNA pellet present in tube after alcohol precipitation	Make sure the isopropanol is ice cold. Precipitation is enhanced by leaving samples in freezer overnight after step.
The extracted DNA is very cloudy	Cellular debris from pellet transferred to tube	Centrifuge sample again and move supernatant to a fresh tube. Take care to avoid pellet.
	Cellular debris not separated from supernatant	Centrifuge sample again. If possible, centrifuge at a higher speed. Move cleared supernatant to a fresh tube.

Appendix A
EDVOTEK® Troubleshooting Guides

PCR AND ELECTROPHORESIS

PROBLEM:	CAUSE:	ANSWER:
There is very little liquid left in tube after PCR	Sample has evaporated	Make sure the heated lid reaches the appropriate temperature.
		If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax (see Appendix B for details)
		Make sure students close the lid of the PCR tube properly.
	Pipetting error	Make sure students pipet 20 µL primer mix and 5 µL extracted DNA into the 0.2 mL tube.
The ladder, control DNA, and student PCR products are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
		Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels.
		The proper buffer was not used for gel preparation. Make sure to use 1x Electrophoresis Buffer.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
After staining the gel, the gel background is very dark.	The gel needs to be destained longer.	Submerge the gel in distilled or deionized water. Allow the gel to soak for 5 minutes.
After staining, the ladder and control PCR products are visible on the gel but some student samples are not present.	Plant DNA sample was not concentrated enough.	Extracting DNA from plants can be challenging.
	Plant DNA sample was degraded.	If DNA is not used right after extraction, store sample at -20°C.
	Wrong volumes of DNA and primer added to PCR reaction.	Practice using micropipets
Some student samples have more/less amplification than others.	Concentration of DNA varies by sample.	There is an inherent variability in the extraction process.
Low molecular weight band in PCR samples	Primer dimer	Low concentration of extracted DNA in PCR reaction.
DNA bands were not resolved.	To ensure adequate separation, make sure the tracking dye migrates at least 3.5 cm on 7 x 7 cm gels and 6 cm on 7 x 14 cm gels.	Be sure to run the gel the appropriate distance before staining and visualizing the DNA.
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™

Appendix B
Preparation and Handling of PCR Samples with Wax

Preparation and Handling of PCR Samples With Wax

ONLY For Thermal Cyclers WITHOUT Heated Lids, or Manual PCR Using Three Waterbaths

Using a wax overlay on reaction components prevents evaporation during the PCR process.

HOW TO PREPARE A WAX OVERLAY

1. Add PCR components to the 0.2 ml PCR Tube as outlined in Module III.
2. Centrifuge at full speed for five seconds to collect sample at bottom of the tube.
3. Using clean forceps, add one wax bead to the PCR tube.
4. Place samples in PCR machine and proceed with Module III.

PREPARING PCR SAMPLES FOR ELECTROPHORESIS

1. After PCR is completed, melt the wax overlay by heating the sample at 94° C for three minutes or until the wax melts.
2. Using a clean pipette, remove as much overlay wax as possible.
3. Allow the remaining wax to solidify.
4. Use a pipette tip to puncture the thin layer of remaining wax. Using a fresh pipette tip, remove the PCR product and transfer to a new tube.
5. Add 5 µl of 10x Gel Loading Buffer to the sample. Proceed to Module IV to perform electrophoresis.

Appendix C
Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

BULK ELECTROPHORESIS BUFFER

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in **Table D**.

Table D — Bulk Preparation of Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 ml		2,940 ml	3000 ml (3 L)

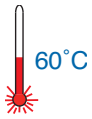
BATCH AGAROSE GELS (1.0%)

Bulk preparation of 1.0% agarose gel is outlined in Table E.

Table E — Batch Preparation of 1.0% UltraSpec-Agarose™

Amt of Agarose	+	50x Conc. Buffer	+	Distilled Water	=	Diluted Buffer (1x)
3.75 g		7.5 ml		367.5 ml		375 ml

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour the appropriate amount of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Proceed with electrophoresis (Module IV) or store the gels at 4° C under buffer.

NOTE:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

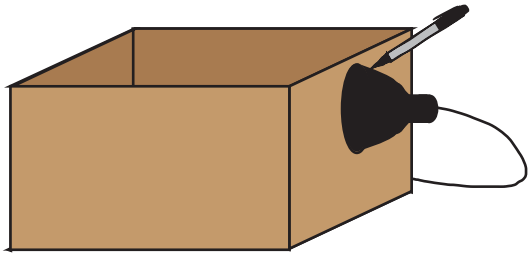
NOTE:
QuickGuide instructions and guidelines for casting various agarose gels can be found our website. **www.edvotek.com/quick-guides**

Appendix D
Creating a Plant Growth Chamber

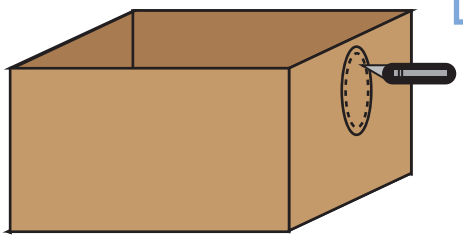
Materials Needed:
- Open top cardboard box (12x12x12 or similar)
- Marker
- Aluminum foil
- Tape
- Reflector lamp
- 23W Compact Fluorescent bulb (CFL bulb)
- Razor or sharp knife

A plant growth chamber can help ensure success with Module I of this experiment.

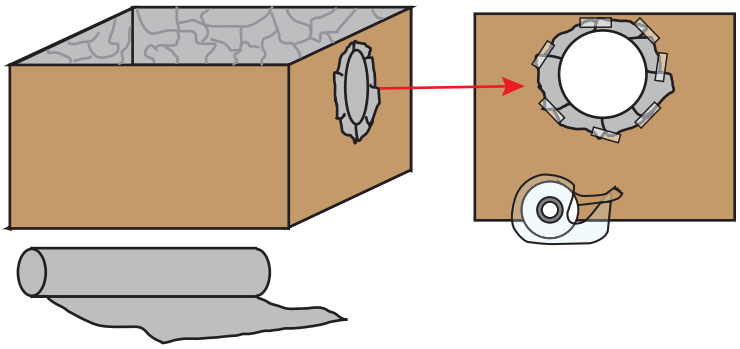
1. Place the reflector lamp on the top center of one of the sides of a small cardboard box. Use a marker or pen to trace a circle around the lamp.



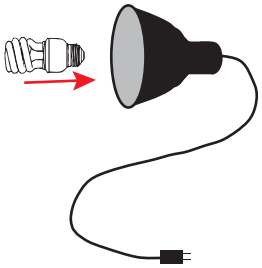
2. Cut a circle 1 cm smaller than the traced circle.



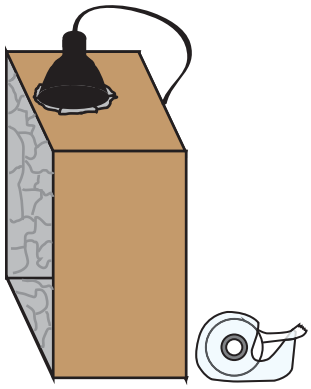
3. Use aluminum foil to line the inside of the box. Use extra pieces to cover the hole. Tape foil in place.



4. Screw the CFL bulb into the lamp.



5. Place the lamp over the hole. Secure with tape.



6. The plant growth chamber is ready to go! Use a small box or riser to adjust the proximity of the potted seeds to the light depending on the seed type and the lighting requirements.