

Genetics of *Arabidopsis thaliana*

Linda J. Robinson

Department of Biology
University of Pennsylvania
415 S. University Ave.
Philadelphia, PA 19104-6018
Linda3@sas.upenn.edu

Abstract: This lab exercise emphasizes hypothesis testing, as students develop and test hypotheses as to the phenotypic ratios that will occur in F2 generation seedlings resulting from a dihybrid cross of *Arabidopsis* plants. Genotypes of the plants are also analyzed for one of the characters. Since both the phenotype and genotype of the same plant are determined for one of the characters, the concept of genetic penetrance is introduced. Students learn that a given genotype does not always result in the predicted phenotype.

Introduction

Arabidopsis is a good model system for studying genetics because the seedlings are easy to grow in the laboratory, the phenotypes are easily observable, the DNA extraction procedure for genotypic analysis is straightforward, and there are a large number of mutants available. This lab exercise emphasizes hypothesis testing, as students develop and test hypotheses as to the phenotypic ratios that will occur in F2 generation plants resulting from a dihybrid cross of *Arabidopsis* plants. Students first determine the phenotypic ratios for two characters, hypocotyl length and presence of trichomes, in seedlings that they planted a week earlier. Then their results are compared to predictions made based on Mendel's laws using the chi-square statistical test. Finally, genotypes of the plants are analyzed for the hypocotyl length character by extracting DNA from the seedlings, using PCR to amplify the alleles, and subjecting the PCR products to gel electrophoresis. Since both the phenotype and genotype of the same plant are determined for one of the characters, the concept of genetic penetrance is also introduced. Students learn that the relationship between genotype and phenotype is not always straightforward, and that a given genotype does not always result in the predicted phenotype.

The objectives of this lab are 1) to understand Mendelian genetics and complete vs. incomplete dominance, 2) to understand hypothesis testing and the chi-square statistical test 3) to develop and test hypotheses for phenotypic ratios in a dihybrid cross, 4) to understand the use of the polymerase chain reaction (PCR) and gel electrophoresis for plant genotyping, and 5) to analyze the genotype of F2 generation plants using the PCR and gel electrophoresis.

This lab exercise was developed five years ago for a first-semester introductory biology course for majors. It's one of the more challenging lab exercises, as many new concepts and techniques are

introduced. It's a multi-week exercise, with portions of the exercise performed over three different three-hour lab periods. A one-week incubation period is required between the first two lab periods. Students plant seeds during the first lab period (requires about 30 minutes), analyze phenotypes, extract DNA, and set up PCR reactions from one-week-old seedlings during the second lab period (requires about 2.5 hours), and analyze their PCR product by gel electrophoresis during the third lab period (requires about 2 hours). All the reagents can be prepared and aliquotted ahead of time, and stored in a freezer.

Student Outline

Background

The genes that you will be analyzing in this laboratory exercise are *CRYPTOCHROME1 (CRY1)* and *GLABROUS1 (GLI)*. Both of these genes are widely expressed in plants indicative of their important functions in plants. The *CRY1* gene codes for the protein cryptochrome1 (CRY1), which is a blue light receptor. Wavelengths of light in the blue and red regions of the spectrum are responsible for all responses of plants to light. The blue and red wavelengths are detected by photoreceptors, proteins within the plant cell plasma membrane. CRY1 is a photoreceptor for blue light. One of the responses to blue light is the regulation of cell elongation during seedling growth. Under normal conditions or under blue light, this process ensures that energy from blue light goes toward growth of new leaf primordia and to lateral growth rather than to elongation of the hypocotyl, the embryonic "stem" below the point where the cotyledons (seed leaves) are attached. Wild type seedlings grown under blue light have shorter hypocotyls than those grown in the dark (see Figure 1, below). We know that this response is regulated by CRY1 since plants with mutations in the *CRY1* gene (*cry1* mutants) have long hypocotyls even when grown under blue light. The *cry1* mutation that you will study in this laboratory is a deletion mutation - a portion of the *CRY1* gene has been deleted.

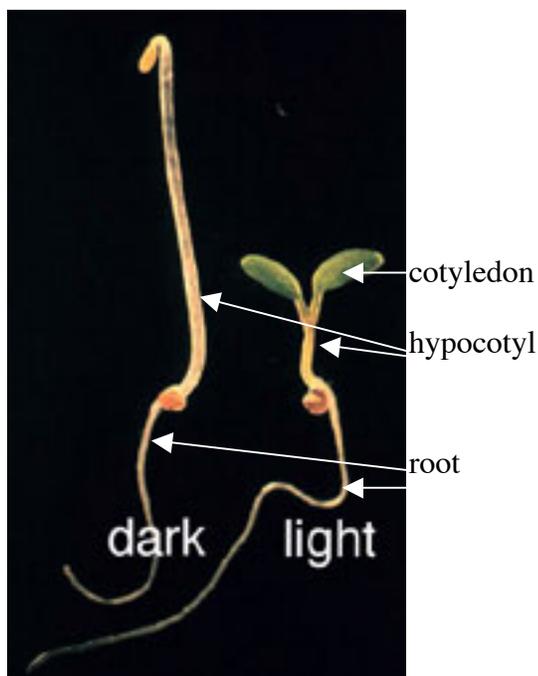


Figure 1: *Arabidopsis* seedlings grown for approximately 7 days in dark versus light. *Arabidopsis* seedling image provided by: Dr. Christain Fankhauser, Universite de Lausanne

The gene *GLABROUS1* (*GL1*) is involved in the pattern of trichome development. Trichomes are single-celled hairs that develop from epidermal cells, and are regularly distributed on the leaves, sepals and stems of plants (see Figure 2, below). Trichomes are believed to play a role in protecting plants from predators and the disease-causing organisms they carry. Only epidermal cells expressing *GL1* will differentiate into trichomes. Mutants with defects in *GL1* (*gll* mutants) do not develop trichomes.

I. Analysis of Phenotype and the Chi-Square Test

In this laboratory exercise, you will undertake a quantitative analysis of the phenotypes expressed in *Arabidopsis* plants as a result of a dihybrid cross. You will be given seeds from F1 hybrid plants. These F1 plants were derived from the crossing of the parental (P1) generation: a wild type (w.t.) *Arabidopsis* plant with a short hypocotyl and trichomes was crossed with a mutant *Arabidopsis* plant with a long hypocotyl, but without trichomes. As indicated above, the *cry1* homozygous mutant plants have longer hypocotyls than the w.t. *Arabidopsis* plants, and the *gll* homozygous mutant plants are lacking trichomes. Your goals are to determine the mode of inheritance and segregation pattern of these two characters by examining the F2 generation. You will plant the seeds, and after a week of seedling growth, count the seedlings on your plate, while identifying each seedling by its phenotype (hypocotyl length and presence or absence of trichomes).

In the case of a monohybrid cross, if the inheritance pattern is complete dominance, we know that the F2 phenotype is represented by a 3:1 ratio. Assuming that the two traits are independently assorted, we can examine each trait separately. For example, we can first score plants only on the basis of whether or not they have trichomes. We can then check our results to see if they conform to a 3:1 ratio. For example, if we count 60 seedlings, we should expect an F2 count of 45 plants with trichomes to 15 seedlings without trichomes. In reality, you may count 37 seedlings with trichomes and 23 seedlings without trichomes. You should then ask whether such a deviation is significant or not. In other words, how often may we expect chance alone to produce a deviation of 8 seedlings in a population of 60 seedlings?

This determination can be made using the chi-square test. First, it must be made clear that the

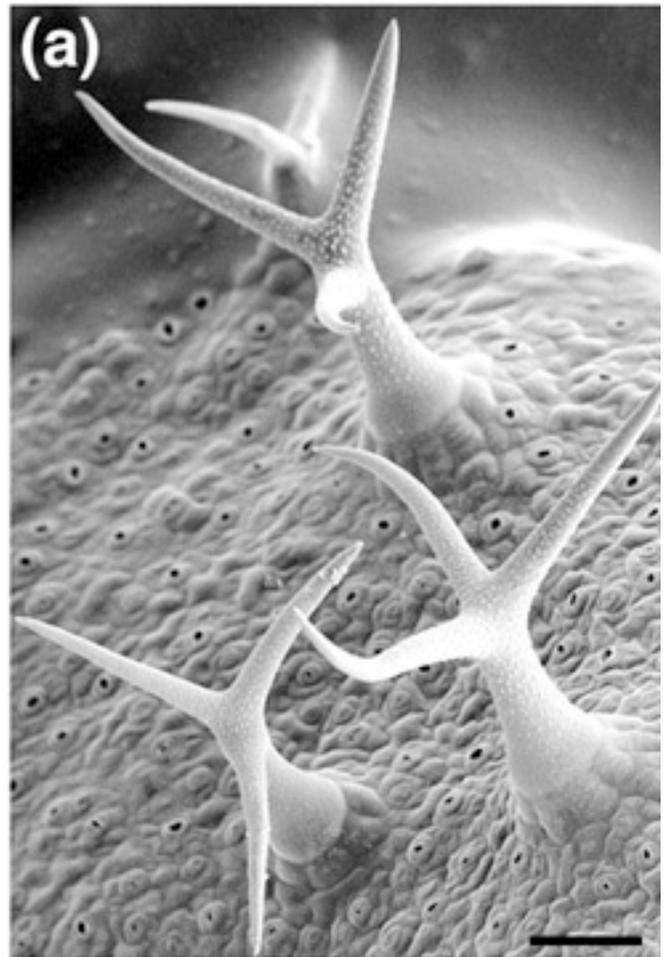


Figure 2: Electron micrograph of trichomes on an *Arabidopsis* leaf. From: Collings, D. A. 2002. Mile-high view of plant biology. *Genome Biology* 2002 3: reports4035.1-4035.3 doi:10.1186/gb-2002-3-11-reports4035

significance of a given deviation is related to the size of the sample studied. If we observe a similar ratio of 5:3 in a sample involving only eight seedlings, and we expect a 3:1 ratio, our results are not all that unexpected. However, if our sample involves 60 seedlings, and we observe a 5:3 ratio, we may begin to question our results. As the sample size increases, the average deviation from the expected fraction or ratio decreases on a proportional basis. Therefore, a larger sample size diminishes the impact of chance deviation on the final outcome.

In genetics it is important to be able to evaluate the significance of observed deviation. When we assume that data will fit a 3:1 ratio, we establish what is called the null hypothesis. It is so named because the hypothesis assumes that there is no real difference between the measured ratio and the predicted ratio. The apparent difference can be attributed purely to chance. Evaluation of the null hypothesis is accomplished by the chi-square test, and on this basis, the null hypothesis may either be rejected or fail to be rejected. If it is rejected, the observed deviation from the expected is not attributable to chance alone. If the null hypothesis fails to be rejected, any observed deviations may be attributed to chance. In this case, the hypothesis is supported.

How far off from our expected ratio will we accept as fitting with our hypothesis? We need to come up with a number that represents the amount of deviation found in our sample from what we expect. First, we need to find the difference between our observed (o) values and our expected (e) values for each group (o-e). Clearly, some of these values may be negative. To avoid this problem, we can square the difference ((o-e)²). Dividing by the expected values will give us the relative deviation from the expected values ((o-e)²/e). Summing these values gives us the value of Chi-squared:

$$X^2 = \sum ((o-e)^2/e)$$

	sample of 8 seedlings		sample of 60 seedlings	
	+ trichomes	- trichomes	+ trichomes	- trichomes
observed (o)	5	3	37	23
expected (e)	6	2	45	15
deviation (o-e)	-1	1	-8	8
(o-e) ²	1	1	64	64
(o-e) ² /e	0.1667	0.5	1.422	4.266
X ² = Σ ((o-e) ² /e)	X ² = 0.6667		X ² = 5.688	

Before testing the significance of our observed deviation, we must first decide on an acceptable false positive rate. The false positive rate is the probability of rejecting the null hypothesis when it is in fact true. Most biologists choose a false positive rate of 0.05. We then consult a “critical values of chi-square” table, shown below. With the chi-square test, the sample size (N) is the number of categories. In our “trichome” example, the number of categories is two (- trichomes and + trichomes). The

“degrees of freedom” is a convention used to account for the number of categories, calculated as the number of categories minus one (N-1). For one degree of freedom, and a false positive rate of 0.05, we observe that the critical X^2 value listed in the table below is 3.84. Our first calculated value of 0.6667 is less than 3.84, so the null hypothesis is supported, and any observed deviations from the expected results can be attributed to chance.

The second calculated X^2 value of 5.688 is greater than the critical value of 3.84. For this sample of 60 seedlings, the null hypothesis is rejected, and the observed deviation from the expected is not attributable to chance alone. After calculating your X^2 , and comparing it to the table value, if your X^2 is greater than the critical X^2 value indicated in the “critical values of chi-square” table for the appropriate false positive rate and degrees of freedom, then the ratio being tested does not fit your data, and the null hypothesis should be rejected. At this point, a new null hypothesis can be generated and tested if necessary.

Critical values of chi-square

Degrees of Freedom	False Positive Rate			
	0.100	0.050	0.010	0.005
1	2.71	3.84	6.63	7.88
2	4.61	5.99	9.21	10.60
3	6.25	7.81	11.34	12.84
4	7.78	9.49	13.28	14.86
5	9.24	11.07	15.09	16.75
6	10.64	12.59	16.81	18.55

II. Analysis of Genotype - Polymerase Chain Reaction (PCR) and Gel Electrophoresis

You will also analyze the genotype of your plant. One method used to analyze genotype is to extract the total DNA from a plant, and then amplify a particular gene of interest using the PCR technique. The PCR product is then run on a gel via the technique of gel electrophoresis, and the size of the DNA segment can thus be determined.

You may have heard news reports of scientists wanting to analyze DNA from mummies, ancient insects preserved in amber, or dinosaur bones. In addition, news about using DNA in determination of paternity, solving rape and murder cases, and identification of victims of the World Trade Center attacks of 2001 are all over the media. All of these are problems that can be solved using the Polymerase Chain Reaction (PCR). PCR amplification is also widely used in plant biology; plant genotyping, gene mapping, diagnostics and diversity assessment all use the technique of PCR. In this lab, you will use PCR for plant genotyping.

PCR was developed by Nobel Prize winner Kary Mullis and associates in 1985, and is essentially

a method that amplifies (replicates) a specific segment of DNA millions of times, while the rest of the DNA is not replicated. The reagents required for amplification are: 1) the original sample, 2) two oligonucleotide primers that form base pairs on opposite sides of the target DNA, 3) a suitable DNA polymerase, 4) the 4 deoxyribonucleoside triphosphates (dATP, dTTP, dCTP, dGTP) and 5) Mg^{2+} ions.

To perform PCR, the reagents are mixed in a thin-walled tube and placed in an instrument called a thermocycler. This machine changes the temperature very quickly according to a set of programmed instructions. The changes in the temperature cause denaturation of the target DNA strands, annealing of primers to the strands, and primer extension (DNA polymerization). These 3 steps constitute a cycle. Many such cycles can be performed quickly to obtain a large number of copies of the target DNA.

For example, to amplify the region of the *CRY1* DNA that contains the mutation resulting in the allele that we're studying, the best results are obtained if the target DNA is taken through 30 amplification cycles as follows:

- Step 1: 30 seconds at 94°C for denaturation
- Step 2: 30 seconds at 60°C for annealing of primers
- Step 3: 60 seconds at 72°C for primer extension (polymerization)

The machine stops after the last cycle and the temperature remains at 4°C until the samples are removed. After 30 cycles, the target DNA produces 2^{30} copies of itself, a sufficient quantity of DNA to use for visualization of the DNA by gel electrophoresis or for sequencing. At least a single copy of the target DNA is required for the PCR to work. PCR is such a sensitive test that, for example, a single hair, a single sperm, or in this case a small portion of a tiny *Arabidopsis* seedling can provide enough material to run the test.

Two important innovations were responsible for automating PCR. First, a heat-stable DNA polymerase was isolated from the bacterium *Thermus aquaticus*, which inhabits hot springs. This enzyme, called "Taq" polymerase, remains active despite repeated heating during many cycles of amplification. Second, DNA thermal cyclers have been developed in which a computer controls the repetitive temperature changes. To familiarize yourself with the technique of PCR, please go to the website: <http://vector.cshl.org/shockwave/pcranwhole.html>, and run the PCR Animation for the "Amplification" and "Amplification Graph" sections.

In this lab, you will amplify a portion of the *CRY1* gene. Based on the results of the gel electrophoresis of the PCR product, you should be able to determine whether one or both of the *CRY1* alleles of your plant are mutant alleles, or whether they are both wild type alleles.

III. Genotype versus phenotype and the concept of genetic penetrance

You will be determining both the genotype and the phenotype of the same plant (for the *CRY1* gene). Remember that genotype refers to a genetic constitution, whereas phenotype refers to the actual expression of a feature through the interaction of genotype, development and environment. For some genes, not every individual with a specific genotype will actually show the phenotype predicted by that genotype. Genetic penetrance is the proportion of individuals with a specific genotype that actually show the phenotype.

For example, there is a dominant allele among humans called "split-hand deformity" (also called "lobster-claw malformation" because of the way it looks). This disease runs in families, and, because it is dominant, it should be seen in every individual that has the gene. In other words, anyone with S-

should have this condition. However, only 70% of people with that genotype actually have “split-hand deformity”. We don’t know why it isn’t in all of them, but it probably has something to do with the interaction of other genes and/or the influence of environmental factors. We say that “split-hand deformity displays 70% penetrance” meaning anyone with the genotype S- has a 70% chance of showing that phenotype. Most of the genes that Mendel initially studied were 100% penetrant. This was fortunate for Mendel because genes with low penetrance can sometimes complicate the determination of mode of inheritance of alleles.

Overview of Laboratory Exercises

Portions of this lab will span 3 weeks. The lab consists of the following procedures:

Part A (Week 1): Each lab group will start by sterilizing and planting seeds from F1 plants on an MS-agar plate. All plates will be placed under grow lights for seed germination and seedling growth.

Part B (Prelab assignment for Week 2): Develop hypotheses as to the expected phenotypic ratios for each character individually (one ratio for hypocotyl length, and a second ratio for presence of trichomes).

Part C (Week 2): Each lab group will score their seedlings by phenotype. The characters to be scored include hypocotyl length and presence of trichomes. Fill out Table 1. Then do the X^2 test for each character to determine whether or not to reject your null hypotheses. Fill out Table 2 and Table 3.

Part D (Week 2): You can now develop a hypothesis to predict the phenotypic ratios from the dihybrid cross, taking both characters into consideration. Complete Table 4. Then do the X^2 test to determine whether or not to reject null your hypothesis. Complete Table 5.

Part E (Week 2): Now you will determine the genotype of a plant. Each student will isolate DNA from one plant, and amplify the *CRY1* alleles using the polymerase chain reaction (PCR).

Part F (Week 3): Run your PCR product on a gel. This analysis should indicate the genotype associated with each phenotype of the hypocotyl length character. Pool your lab section’s results for genotypic analysis and fill out Table 7.

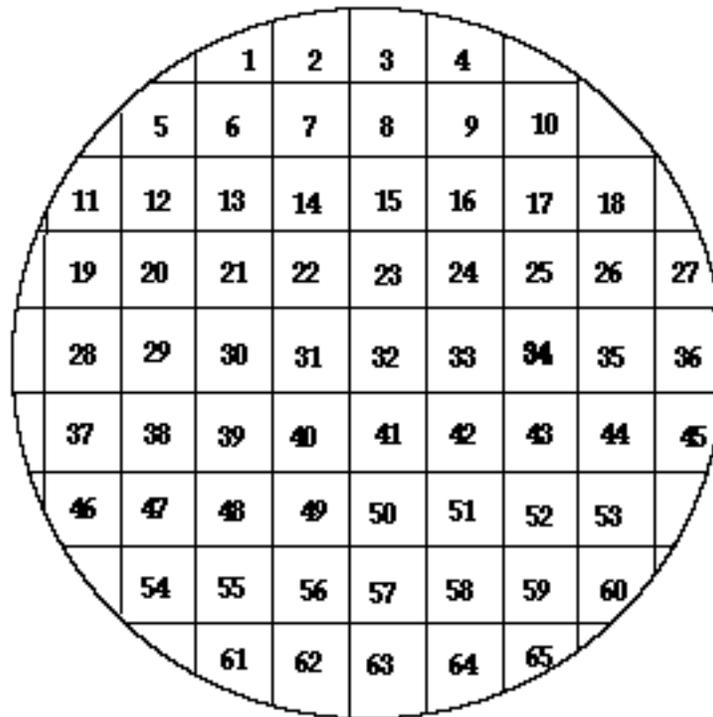
For Part A, seed sterilization and planting, you will be working in groups of 2 or 3, such that each lab section should have 8 groups. For Part B, the prelab assignment, each student should work independently. For Parts C and D, analysis of phenotypes, you will be working in groups of 2 or 3. For Part E, extraction and PCR analysis of plant DNA, each student will work independently. For Part F, gel electrophoresis, up to 8 students will share each gel (4 gels per lab).

Procedure

Part A (Week 1): Planting of Arabidopsis Seeds

1. Using a sterile transfer pipet, transfer about 1 ml (fill the tube) of 70% ethanol to the tube containing the seeds. Cap the tube and invert it five times to mix. Allow the seeds to settle to the bottom of the tube.
2. Using the same transfer pipet, remove as much of the ethanol as possible without removing many of the seeds. The ethanol can be discarded in the sink.

3. Using a new sterile transfer pipet, add about 1 ml of sterile water to the tube containing the seeds. Cap the tube and invert it five times to mix. Allow the seeds to settle to the bottom of the tube.
4. Using the same transfer pipet, remove as much of the water as possible without removing many of the seeds. The water can be discarded in the sink.
5. Repeat steps 3 and 4 again, washing the seeds with a second ml of water.
6. Using a new sterile transfer pipet, add about 1 more ml of sterile water to the tube containing the seeds.
7. Using the 10-ml pipet, add 15 ml of sterile water to an empty Petri dish. Cap and invert the tube with the seeds to resuspend the seeds in water. Making sure that the seeds are resuspended well in the water, open the cap and quickly pour the water and seeds into the Petri dish with the water.
8. Label the side of an MS agar plate with your group name, your TA name, and your lab section, day and time, and place the MS agar plate over the grid provided below.
9. Remove the lids from both the plate with seeds, and the MS agar plate. Set a p20 pipetman to 5 ml, and attach a pipet tip. Push down on the plunger to the first stop. Guide the end of the pipet tip into the plate with the seeds. Position the pipet tip over one of the seeds, and release the plunger, so that you suck up a single *Arabidopsis* seed into the pipet tip, along with 5 ml of water. Now position the pipet tip over the surface of the agar in the section of your MS plate represented by square #1 shown on the grid below, showing through your plate. Dispense the contents of the pipet tip, including a single seed and 5 ml of water, onto the surface of the agar in the section of your MS agar plate represented by square #1. Watch the demonstration by your TA.
10. Repeat this procedure to plate a single *Arabidopsis* seed in each square of the grid below, and replace the lid on your plate. Put the plate in the black container to grow for one week under lights.



or tall, compared to the other seedlings on the plate, and fill in the “hypocotyl length” section of Table 1. Score the remaining seedlings on the plate for hypocotyl length, filling in Table 1. **If you did not see leaf primordia on a seedling, do not score the seedling for hypocotyl length - skip this seedling entirely.**

Table 1: Phenotypic Analysis of *Arabidopsis* seedlings

Seedling No.	Trichomes (+ or -)	Hypocotyl Length (S, M, or T)	Seedling No.	Trichomes (+ or -)	Hypocotyl Length (S, M, or T)	Seedling No.	Trichomes (+ or -)	Hypocotyl Length (S, M, or T)
1			21			41		
2			22			42		
3			23			43		
4			24			44		
5			25			45		
6			26			46		
7			27			47		
8			28			48		
9			29			49		
10			30			50		
11			31			51		
12			32			52		
13			33			53		
14			34			54		
15			35			55		
16			36			56		
17			37			57		
18			38			58		
19			39			59		
20			40			60		

- Fill in the total number of seedlings that you analyzed in the first row of Table 2.
- To calculate the “expected” number of seedlings of each phenotype, multiply the total number of seedlings by the appropriate ratio from your Prelab Assignment (Part B above). Using the example of Mendel’s peas, if you expect a ratio of 3 round:1 wrinkled pea, you would multiply the total number of peas by 3/4 to obtain the “expected” value for round peas, and by 1/4 to obtain the “expected” value for wrinkled peas. Do this for the “hypocotyl length” trait and the “presence of trichomes” trait, and fill in the appropriate numbers in the “expected” row of Table 2.
- Count the observed number of seedlings of each phenotype (+ trichomes, - trichomes, short hypocotyl, medium hypocotyl, and tall hypocotyl), and fill in the “observed” row for each category in Table 2.
- For each category individually (for each column of Table 2), calculate the deviation $(o-e)$, $(o-e)^2$, and $(o-e)^2/e$, and fill in Table 2. Calculate X^2 for both the “trichome” character and the “hypocotyl length” character.

Table 2: X^2 Analysis

	sample of _____ seedlings				
	+ trichomes	- trichomes	short hypocotyl	medium hypocotyl	tall hypocotyl
observed (o)					
expected (e)					
deviation (o-e)					
$(o-e)^2$					
$(o-e)^2/e$					
$X^2 = \sum((o-e)^2/e)$	$X^2 =$		$X^2 =$		

9. Determine the degrees of freedom for both characters, and fill in Table 3 below. Remember that the degrees of freedom represent the number of categories minus one. Then go to the “critical values of chi-square” table in the “Background” section of this chapter. From your calculated values of X^2 in Table 2 for each character, use the “critical values of chi-square” table to determine whether or not to reject the null hypothesis. Use a false positive rate of 0.05 for this analysis.

Table 3

Degrees of Freedom	Reject Null Hypothesis?
-------------------------------	------------------------------------

“trichome” character

“hypocotyl length” character

Part D (Week 2)- Chi-square Test for the Dihybrid Cross:

1. The next step is to develop a hypothesis as to the expected phenotypic ratio for the dihybrid cross, taking both characters into consideration. Fill out Table 4 below.

Table 4: Expected Phenotypic Ratios for the Dihybrid Cross

<u>Phenotype</u>	<u>Expected Ratio (show your math)</u>
short hypocotyl + trichomes	
short hypocotyl - trichomes	
medium hypocotyl + trichomes	
medium hypocotyl - trichomes	
tall hypocotyl + trichomes	
tall hypocotyl - trichomes	

2. To calculate the “expected” number of seedlings of each phenotype, multiply the total number of seedlings by the appropriate ratio from Table 4. Fill in the appropriate numbers in the “expected” row of Table 5 below.

Table 5: χ^2 Analysis of the Dihybrid Cross

	sample of _____ seedlings					
	short + trichomes	short - trichomes	medium + trichomes	medium - trichomes	tall + trichomes	tall - trichomes
observed (o)						
expected (e)						
deviation (o-e)						
$(o-e)^2$						
$(o-e)^2/e$						
$\chi^2 = \sum((o-e)^2/e)$	$\chi^2 =$					

- Count the observed number of seedlings of each phenotype (short + trichomes, short - trichomes, medium + trichomes, etc) for all 6 phenotypes, and fill in the “observed” row for each category in Table 5.
- For each category individually (for each column of Table 5), calculate the deviation $(o-e)$, $(o-e)^2$, and $(o-e)^2/e$, and fill in Table 5. Calculate X^2 by adding all of your values for $(o-e)^2/e$.
- Determine the degrees of freedom, and go to the “critical values of chi-square” table to determine whether or not to reject the null hypothesis. Fill in Table 6. Use a false positive rate of 0.05.

Table 6:**Degrees of Freedom:****Reject Null Hypothesis?***Part E (Week 2) - DNA Extraction From Arabidopsis Seedling and PCR:*

In this part of the experiment, you will first extract DNA from an *Arabidopsis* seedling. You will then set up a PCR reaction by mixing some of this DNA with nucleotides, *CRY1* primers, and Taq polymerase. The primers that you will use are complementary to sequences on either side of a 784 bp region of the wild type *CRY1* gene. This is the portion of the *CRY1* gene that contains the 452 bp deletion in the *cry1* mutant. Therefore, the PCR reaction should amplify the two alleles from your plant, and, following gel electrophoresis of the PCR product, you should be able to determine your plant's genotype for this character (*CRY1/CRY1* (CC), *CRY1/cry1* (Cc), or *cry1/cry1* (cc)).

The class will randomly assess the genotypes of the population of F2 *Arabidopsis* seedlings as follows. One group's plate will be chosen, and one student in the lab will extract DNA, and determine the genotype, from the first seedling on the chosen plate. If leaf primordia were not visible on this seedling, take the second seedling instead. The second student in the class will extract DNA from the second seedling. If leaf primordia aren't visible, take the third seedling instead. Continue in this manner until every student in the class has extracted DNA from a seedling on the chosen plate. **Make sure to note the phenotype when choosing your seedling!**

- Choose the seedling that you will remove for DNA extraction, according to the directions above. **Note your plant's phenotype for hypocotyl length (S, M, or T):** _____
Gently remove this seedling, including the roots, from the agar plate using forceps. Transfer the seedling to a sterile 2-ml microfuge tube, placing the seedling near the bottom of the tube.
- Add 100 ml of Extraction Solution to your seedling in the microfuge tube. Close the tube and vortex briefly. Make sure the seedling is covered by Extraction Solution (you may need to push it down with forceps).
- Incubate in a heating block set to 95 °C for 10 minutes.
- Add 100 ml of Dilution Solution and vortex to mix.
- Obtain a thin-walled 0.6-ml PCR tube from the lab cart. Label the TOP of this tube with your initials. Add the reagents as indicated in the table below to the tube, **using a new sterile tip for each reagent**. The “Extracted *Arabidopsis* DNA” is the contents of the tube containing the *Arabidopsis* seedling that you boiled in order to extract the DNA. Add the **solution** in this tube, **not**

part of the seedling itself. The two primers and the PCR Reaction Mix are on the ice bucket in the center of each table. Make sure to add both primers. The PCR Reaction Mix contains dATP, dTTP, dGTP, dCTP, Taq polymerase, and loading dye for electrophoresis.

Reagent	Volume
Extracted Arabidopsis DNA	4 ml
Primer # 1501	1 ml
Primer # 2221	1 ml
Sterile water	4 ml
PCR Reaction Mix	10 ml

- Mix the contents of the tube by tapping and microfuge for about 10 seconds using the special 0.6 ml tube adaptors for the microfuge. Use another student's sample to balance the microfuge. **Make sure to label the top of the tube with your initials or a symbol that designates your tube.**
- When indicated by your TA, place your tubes in the thermal cycler. Your TA will give a brief demonstration of how the machine works. The thermal cycler has already been programmed to run as follows:

Cycles	Action	Temperature	Duration
1	Denaturation	94°C	3 minutes
2-30	Denaturation	94°C	30 seconds
	Annealing	60°C	30 seconds
	Primer Extension	72°C	60 seconds
31	Hold	4°C	?

The 30 cycles will be complete in about 2 hours. Your TA will remove your lab's tubes from the thermal cycler and store them at 4°C until the next lab session.

Part G (Week 3) - Gel Electrophoresis of PCR Product:

1. Up to 4 gels should be prepared per laboratory, and up to 8 students can share a single gel. Therefore, each group of six to eight students will pour a 1.16% agarose gel. Obtain a complete Pharmacia minigel apparatus and power supply, and make sure that the parts are clean. Watch the demonstration by your T.A.
2. Weigh out 0.7 g agarose, and add it to a clean 250-ml flask. Obtain a 500 ml beaker containing 1X TBE. Carefully measure 30 ml of 1X TBE using a 100-ml graduated cylinder. Add this 30 ml of TBE to the agarose in your 250-ml flask, and mix by swirling gently. Place the 250-ml flask containing the agarose and TBE in the microwave oven and heat for 60 seconds. **CAUTION: Use extreme care when handling boiling solutions of agarose; they can cause severe burns!! Wear the thermal protective gloves provided when handling the hot beaker of agarose!** Take the flask out, using the gloves provided, and mix by swirling gently, without making bubbles. Continue to mix by swirling until agarose particles are no longer visible.
3. Add 30 ml more of room temperature 1X TBE to the 250-ml flask of melted agarose and swirl to mix. Seal the ends of your gel tray with tape to make a container, and place the tray on the benchtop. Place the 11-well comb in the designated slots at one end, and pour the agarose from the 250-ml flask into the tray. **Do not move or touch the tray until the agarose has solidified and turns opaque in color (at least 15 min).**
4. When the agarose has solidified, gently remove the tape and the comb. Lower the tray into the flat surface of the apparatus, such that the wells line up over one of the red stripes on the white plate. Slowly pour 1X TBE into the apparatus until the buffer compartments are full, and the gel is covered by about 1 mm of liquid. The gel is now ready to be loaded with your samples.
5. The "PCR Reaction Mix" already contains loading dye for electrophoresis, so there is no need to add additional dye. Each group of six to eight students should also obtain the following DNA samples from their TA:
 - a tube containing marker DNA (loading dye has already been added)
 - two tubes which will represent the "controls" for the experiment:
 - a PCR reaction from a homozygous wild type (*CRY1/CRY1*) plant - labeled CC
 - a PCR reaction from a homozygous mutant (*cry1/cry1*) plant - labeled cc

If necessary, microfuge the tubes for about 15 seconds to ensure that all of the liquid is at the bottom of the tube (be sure to balance microfuge).

6. Load 10 ml of each sample in the gel slots as demonstrated by your TA. Make sure to take note of the sequence of the samples in the gel wells. Since up to 8 students will be loading their samples onto a single gel, make sure to note the well number in which each student's sample is loaded. Each gel should contain the following samples:

Lane #	Sample	Volume Loaded
1	Standard marker DNA	10 ml
2	wild type control (<i>CRY1/CRY1</i>) PCR reaction (CC)	10 ml
3	mutant control (<i>cry1/cry1</i>) PCR reaction (cc)	10 ml
4-11	student PCR reactions	10 ml (only 1/2 of reaction)

Put the top on the apparatus, and connect the electrodes to the power supply. Two gels can be plugged into each power supply. Make sure that the black electrode (cathode) is in the back (behind the wells), and that the red electrode (anode) is in the front (the DNA will run TOWARD the anode). **CAUTION: Before turning on the power supply, make sure that the apparatus is closed and well shielded, that the electrodes are connected to the power supply, and that there are no exposed parts that could impart an electric shock!** Turn the power supply on by pressing the central button. Set the voltage to 100 volts by pressing the “Set” button until the volts light comes on, and using the arrows to set the voltage to 100. Press the “Run” button to begin electrophoresis.

7. While the gel is running, keep an eye on the migration of the red band (the phenol red). It should migrate toward the anode. If it's migrating toward the cathode, turn the power supply off, and check that the electrodes are connected correctly. After 40 min, the red band should have migrated about 1/2 the length of the gel. Turn off the power supply by hitting the run button. **Do not remove the lid of the electrophoresis chamber while the run light is illuminated!!**
8. After the gel has run for 40 min, prepare a solution of SYBR Gold. First, put on latex gloves. **WARNING: Although the Ames test has shown low toxicity for SYBR Gold, we still caution you to wear gloves and pay special attention to safety methods since any intercalating agent is hazardous by nature.** Carefully measure 50 ml of 1XTBE into your 100 ml graduated cylinder, and add it to a staining container. Then add 5 ml of the 10,000X SYBR Gold solution on the TA's cart to the 50 ml of 1XTBE in the staining container. Do not put the 10,000X SYBR Gold solution on ice, or it will solidify. Gently mix the SYBR Gold solution in the staining dish by swirling.
9. Wearing latex gloves, carefully remove the gel tray from the apparatus. Use caution and hold the gel on the tray, as the gel will be slippery. Transfer the gel to the SYBR Gold solution in the staining tray by gently sliding it off the gel tray into the staining tray. Stain for at least 10 min, occasionally agitating the tray to mix the SYBR Gold solution over the gel.
10. Carry the staining tray to the photography cart, and carefully transfer the gel onto the UV lightbox. Make sure the UV shield is down before turning the UV light on. Take a picture of your gel as demonstrated by your TA. Print out a picture for every student who has loaded their PCR reaction on the gel.
11. Each student should write their plant's phenotype (S, M, or T hypocotyl length) and genotype on a table on the blackboard. The genotype indicates whether the alleles for the *CRY1* gene are homozygous wild type (*CRY1/CRY1*), homozygous mutant (*cry1/cry1*), or heterozygous (*CRY1/cry1*). Let's use the nomenclature cc for homozygous mutant, Cc for the heterozygous, and CC for homozygous wild type. Copy the data from the board and fill in Table 7 below.
12. Were all of the PCR results obtained for genotype the expected results based on the phenotypic analysis? _____

If not, why do you think the phenotypes and genotypes don't always correlate?

Notes for the Instructor:

Seed Planting:

Although the seed planting is tedious, most students find it easier than they thought it would be once they get going. We have students work in pairs and "trade off" seed planting in order to plant about 60 seeds in about 15 minutes. The grid is provided so that the seedlings will be separated, making them easier to analyze. However, seedlings are still analyzable if two seeds are placed within a few of the squares on the grid, or if the seeds are not placed in the middle of the grids. Students shouldn't worry if they end up with several seeds in the same square, or if their seeds are not perfectly in the middle of each square of the grid.

Students should try to keep their MS agar plates, seeds, and pipet tips as sterile as possible during seed planting to avoid mold contamination. Approximately 25% of the student plates with seeds end up with some mold after the one-week incubation under grow lights. However, in most cases the mold is restricted to a small area of the plate, and at least half of the seedlings on a moldy plate are usually analyzable. An ABLE participant suggested inclusion of nystatin in the MS agar medium to inhibit mold growth.

Seedling growth:

Through trial and error, we found that the temperature, light conditions, and growth media are critical to obtaining seedlings at the correct stage of development after one week. The current conditions that we are using are working well, and are described in Appendix B.

Incomplete dominance, intermediate phenotypes and subjective observations:

For the *CRYPTOCHROME1* gene, there is incomplete dominance where the F1 hybrids have an intermediate phenotype. We have intentionally chosen a gene that displays incomplete dominance in order to teach students that the relationship between genotype and phenotype is rarely as simple as Mendel found with most of his pea plant characters, which displayed complete dominance. We also want to show students that despite the incomplete dominance shown by the *CRY1* gene, the basic principles of segregation and independent assortment still apply.

The intermediate phenotype in this case is hypocotyl length. Since there is always a spectrum of hypocotyl lengths observed, students have difficulty deciding where to "draw the line" between small, medium, and tall hypocotyls. Sometimes students use rulers to measure hypocotyl length, and then decide on "cut-offs" for their categories. However, since the students are expecting a 1:2:1 phenotypic ratio, their observations and their decisions on where to draw the line are often biased by this knowledge. They tend to play with the numbers to try to make their data fit this ratio. Therefore, we try to emphasize to students that their observations should not be biased by this knowledge, and that it is in fact unethical to try to make the data fit the hypothesis. For this reason, we stopped giving the students rulers, and now encourage them to arbitrarily decide on their three phenotypic characters based on eyeballing the height of the seedlings. We emphasize the problems with such a subjective approach, and feel that this is also a lesson in real-life genetics. Often researchers have to make subjective estimate of a phenotype, and this is the case for the hypocotyl length character.

DNA extraction, PCR, and gel electrophoresis:

It is important that students choose their seedling for DNA extraction randomly. We found that despite instructions to choose a seedling randomly (for example, TAs told students to choose seedlings

number 10 and 20 on their plates), students tended to choose a disproportionate number of tall plants over short and medium seedlings. When we then calculated the genotypic ratio across the entire (200 student) class, and performed the chi-square test for genotype, we found that the null hypothesis was often rejected. When seedlings are chosen randomly, the chi-square test for genotype is almost always supported. By choosing a single plate of seedlings, and having all of the students in a lab section choose a seedling for DNA extraction from a single plate, the seedling choice seems to be more random. We try to have students pass the plate of seedlings around the lab, and pull their seedling out of the plate in order as the plate goes around the lab.

An alternative method for extracting plant DNA to the Sigma kit is provided in Appendix D. This method will save money, as the Sigma kit would not need to be purchased. If this method is used for DNA extraction, a "home-made" PCR reaction mix can be generated by a less-costly method of mixing appropriate concentrations of Taq polymerase and dNTPs.

The biggest problem with the PCR and gel electrophoresis is pipetting errors. If students use the micropipettors correctly, they will usually see the appropriate band(s) on their gel. In a previous lab, we teach students correct use of micropipettors, and they get practice in using them, and in loading gels. Despite this instruction and practice, about 25% of students do not obtain band(s) on the gel following electrophoresis of their PCR product due to improper use of the micropipettors.

Genetic Penetrance

In the analysis of seedling phenotypes, the chi-square analysis for the hypocotyl length character, but not for the trichome character, often results in rejection of the null hypothesis. This occurs for two reasons; the subjective nature of the hypocotyl length analysis due to the continuum of phenotypes and the low penetrance displayed by the *CRYPTOCHROME1* gene. On the other hand, the *GLABROUS1* gene, which controls trichome development, displays high penetrance, and phenotypic analysis is much more straightforward.

For large classes (250 students) with many lab sections, we collect corresponding phenotype and genotype data for the hypocotyl length character from the entire class and pool the data. We have students do a chi-square analysis for phenotype and genotype separately from the pooled data, and compare them. Whereas the null hypothesis for phenotype is often rejected, the null hypothesis for genotype is usually supported by the data. This exercise illustrates the concept of genetic penetrance to the students with an example of a gene that exhibits low penetrance.

Assessment

Results from student evaluations of this laboratory exercise are included in Appendix E.

Materials

For a lab section of 20 students (2 or 3 students per group; 8 groups per lab section):

Equipment:

- P20 digital micropipettors: 8
- P200 digital micropipettors: 8
- grow lamps: 1 cart (see Appendix B)
- dissecting microscopes or magnifying glasses: 8
- vortexers: 3
- heating block set to 95°C or boiling water bath with floating tube holder: 1
- microfuge: 1
- thermal cycler: 1
- gel apparatus for electrophoresis: 4
- power supply: 2
- balance: 1
- microwave: 1
- gel photography equipment: 1

Supplies:

- Arabidopsis seeds (see Appendix A): 8 X 0.6 ml tubes containing approximately 100 seeds each
- 70% ethanol: 8 flasks of 50 ml each
- sterile water: 8 flasks of 100 ml each
- sterile transfer pipets: 32 (see Appendix C)
- pipet pumps for 5 or 10 ml pipets: 8
- sterile 10 ml pipets: 8
- sterile 100 mm Petri dishes: 8
- MS agar plates (see Appendix C): 8
- Sharpie markers for labeling: 8
- "large orifice" p20 pipet tips: 8 boxes (see Appendix C)
- sterile p20/p200 pipet tips: 8 boxes
- black plastic trays for growing seedlings: 1 (see Appendix B)
- forceps: 8
- sterile 2 ml microfuge tubes: 20
- 0.6 ml PCR tubes: 20
- Extraction Solution (from Sigma XNAP kit; see Appendix C): 3 X 1 ml tubes
- Dilution Solution (from Sigma XNAP kit; see Appendix C): 3 X 1 ml tubes
- *CRY1* primer #1501 (10 pmoles/ml; see Appendix C): 3 X 10 ml (in 1 ml tubes)
- *CRY1* primer #2221 (10 pmoles/ml; see Appendix C): 30 X 10 ml (in 1 ml tubes)
- PCR Reaction Mix (from Sigma XNAP kit; see Appendix C): 3 X 100 ml (in 1 ml tubes)
- Sterile water for PCR: 3 X 100 ml (in 1 ml tubes)

Acknowledgements

Dr. Scott Poethig, a professor and plant geneticist in the Biology Department at the University of Pennsylvania, provided the idea for this laboratory exercise, the *Arabidopsis* seeds, the initial set of PCR primers, editing of the original lab manual chapter, and considerable help and expertise in the development of the exercise. Thanks to Dr. Christian Fankhauser, Universite de Lausanne, for allowing the use of the *Arabidopsis* image in Figure 1 from his website. Yelena Cherkashina and Svetlana Kozik helped with the testing of the lab and the preparation of laboratory materials. Glenn Doyle helped with primer design and editing of the original lab manual chapter. The participants of the ABLE 2006 workshop contributed a number of helpful suggestions.

Literature Cited

Collings, D. A. 2002. Mile-high view of plant biology. *Genome Biology* 2002 3: reports4035.1-4035.3
doi:10.1186/gb-2002-3-11-reports4035

Handling *Arabidopsis* Plants and Seeds, Methods used by the *Arabidopsis* Biological Resource Center,
<http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/handling.html>

About the Author

Linda Robinson received her B.S. in Biology from Bucknell University (Lewisburg, PA) and Ph.D. in Molecular Cell Biology from Washington University (St. Louis, MO). She is currently an Instructional Laboratory Coordinator and Lecturer in the Biology Department at the University of Pennsylvania, where she teaches introductory biology. Although her background and research training is in cell biology (membrane trafficking in eukaryotic cells), she spends most of her time preparing and developing labs for introductory biology courses. She also teaches introductory biology and animal physiology lectures and is responsible for TA training and organization of the large-enrollment introductory biology courses and their labs.

© 2007 University of Pennsylvania

Appendix A: Obtaining the seeds

The seeds given to the students in this lab are from F1 hybrid plants. These F1 plants are derived from the crossing of the parental (P1) generation: a wild type (w.t.) *Arabidopsis* plant with a short hypocotyl and trichomes (CCGG) is crossed with a mutant *Arapidosis* plant with a long hypocotyl and without trichomes (ccgg). Following this cross, the resulting seeds are collected and planted. The resulting F1 plants are grown and allowed to self-pollinate. The seeds from these F1 hybrids are collected and stored in 1.5-ml microfuge tubes in a dessicator in the refrigerator. They are viable when stored this way for at least two years. Seeds from approximately 8-10 plants (one 1.5 ml tube-full) are required for a class of 250 students.

The CCGG wild type seeds should be of the "Columbia" ecotype. The ccgg mutant seeds will soon be available from the *Arabidopsis* Information Resource (ABRC) and seed bank at Ohio State University. These seeds can be ordered through the TAIR, the *Arabidopsis thaliana* information resource center online at <http://www.arabidopsis.org>.

The crossing of the P1 generation plants takes some expertise, and is not recommended for an instructor who has never done this before, and who does not have access to an *Arabidopsis* researcher from whom they can learn. In this case, I can ship a small number of F1 hybrid seeds, and the heterozygotes can be selected and propagated as follows.

Isolating heterozygous (Cc) seeds:

I will supply a small number (100 or so) seeds produced by the F1 heterozygotes (these are the seeds PRODUCED BY the F1 heterozygotes, so these seeds will become the F2 generation plants following germination). These are the seeds that the students usually plant for the laboratory exercise, but this number of seeds (100 or so) will not be enough for all of your students! Therefore, you can follow the following procedure to generate more of these seeds:

1. Plant about 8 seeds per pot in two 10 cm square pots (16 seeds total) in soil. Allow the plants to grow until they begin flowering.
2. Remove one leaf from each of the 16 plants without disturbing the remainder of the plant.
3. Extract DNA and set up PCR reactions to amplify the CRY1 alleles as described in the "Genetics of *Arabidopsis*" lab exercise. Just substitute the leaf for the seedling and follow the procedure in the lab exercise.
4. Run gels on the PCR products to determine the genotype of each of the 16 plants for the CRY1 character.
5. Throw away all plants that are homozygous (either CC or cc). About half of the plants should be heterozygous (Cc), and these are the ones that you should keep. Continue to grow these plants until they flower and self-pollinate (they will do this without your interference).
6. Allow the heterozygous plants to go to seed. When seed pods (siliques) are fully formed, allow them to dry for about a week. Then harvest seeds from the plants. Make sure to harvest seeds from each plant individually, keeping each plant and its seeds separate from the other plants and their seeds. Do not contaminate seeds from one plant with seeds from another plant. Label the seed stocks, and store them in 1.5 ml tubes. You should obtain about 1000 seeds from each plant.

Progeny Test of Cc plants for Gg seeds:

7. For each of your different Cc seed stocks (you should have approximately 8), sterilize and plant about 32 seeds onto an MS agar plate, following the instructions in the "Genetics of *Arabidopsis*" lab exercise. Make sure to label these plates with the number of the seed stock that they came from (devise your own numbering system). Also make sure to keep the 8 or so tubes of seed stocks dry and refrigerated until you get the results from the progeny test.
8. Allow all the planted seeds to germinate and grow on the MS agar plates under grow lights until the plant is large enough to analyze the leaves for trichomes (1-2 weeks).
9. Analyze the 32 seedlings from each seed stock under a dissecting microscope to determine whether or not each of the 32 seedlings (from each seed stock) have trichomes.
10. Select and use the Cc seed stocks that give you a 3:1 ratio (trichomes:no trichomes). For 32 plants, this ratio should be approximately 24:8. As long as you get most plants with trichomes and at least four or five plants without trichomes, you can be pretty sure that the Cc seed stock is also Gg (heterozygous for both characters). If all 32 plants have trichomes, the seed stock is GG, and you can throw it away. If all 32 plants do not have trichomes, the seed stock is gg, and you should throw it away. About half of the seed stocks (approximately 4 out of 8) should give rise to progeny that give a 3:1 ratio (3 trichomes:1 no trichomes).

Generation of seeds for student use:

11. Throw away all the plants from the progeny test, but be sure to save the four or so 1.5 ml tubes containing the CcGg seeds (about 1000 seeds/tube). Plant the seeds from these CcGg seed stocks. We plant about 10 seeds for each class of approximately 250 students. These will become the CcGg heterozygous F1 plants. Allow these plants to grow, self-pollinate, and go to seed. Collect these seeds and give them to the students for the laboratory exercise. About one 1.5-ml tube full of seeds (from about 10 plants) is required for a class of 250 students (about 125 plates).

Notes:

- Instructions for "Handling *Arabidopsis* Plants and Seeds" can be found on the ARBC website at: <http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/handling.htm>
- Since the life cycle of *Arabidopsis* takes about two months (from seed to seed again upon self-pollination) the first part of the above procedure (steps 1-6) will take about two months. The genotyping for the CRY1 character only takes a couple of days.
- Since plants do not have to be mature in order to analyze them for trichomes, the "progeny test" (steps 7-10) can be done in 1-2 weeks.
- Although the above procedure therefore takes about 3 months, about 4000 seeds should be generated by this procedure (about 1000 per plant).
- Only about 8 of the 4000 or so CcGg seeds are required per semester. Therefore the above procedure should produce enough CcGg seeds to last forever.
- Remember that once the above procedure (steps 1-10) is completed, a two-month period is still required (we do this every summer) to generate seeds for student use (step 11).

Appendix B: Growth of *Arabidopsis* seedlings

Instructions for "Handling *Arabidopsis* Plants and Seeds" can be found on the ARBC website at: <http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/handling.htm>

Students plant seedlings onto MS agar plates. Preparation of MS agar plates is covered in Appendix C. Once the seeds have been planted, the plates should be placed in a black or dark-colored container such as bottom section of the trays used by plant nurseries. Alternatively, the surface of the shelf could be covered with a black or dark piece of paper that does not reflect light.

We grow seedlings on "grow carts" on wheels, which are placed in the teaching laboratories. *Arabidopsis* seedlings grow best at 22-25°C, or "room temperature", so it is important that the temperature in the room where they grow is regulatable and consistent. The "galvanized shelf truck" (48" X 24" X 80") that we use as a growth cart is from Global Industrials (www.globalindustrial.com), catalog #489057. The cart has four adjustable shelves; three of which the MS plates with seeds are placed, and the fourth is used to hang lamps for the third (top) shelf of plates. We can fit MS plates from 4 lab sections on each shelf (12 lab sections per cart), so a single cart can accommodate up to 240 students (12 lab sections of 20 students each). The lamps, ballasts and bulbs we use are from K-B Lighting (2101 Byberry Rd., Philadelphia, PA 19116). We use 3 wire cord/delete lens/(2), Sylvania QT 232, 120 V (pull chain) lamps plus ballasts and four F032T8 841 fluorescent bulbs per shelf. The lamps are hung via chains from the shelf above, such that the fluorescent light bulbs are 17 inches above the plates of seeds. The seedlings are grown under constant light (lamps remain on; day and night) for one week.

Appendix C: Reagents and Supplies

MS agar plates

One liter prepares approximately 40 plates (about 25 ml per plate). For one liter, prepare as follows:

1. Dissolve 2.15 g of MS powder (Murashige and Skoog Basal Salt Mixture; Sigma catalog #M5524) in 800 ml of water in a 2L flask with a stir bar and mix on stir plate.
2. Adjust pH to 5.7 with 1M KOH while mixing.
3. Add 10 g of sucrose, and mix to dissolve.
4. Add 8 g of agar (such as Bacteriological Agar from Sigma; catalog #A-5306)
5. Adjust volume to 1L with water.
6. Autoclave on liquid cycle for 30 minutes.
7. Cool to 50°C and pour approximately 25 ml per plate into sterile 100 mm Petri dishes.
8. Allow to cool and solidify overnight.
9. Store at 4°C for as long as a few months if necessary.

Sterile transfer pipets for sterilizing seeds: Fisher Scientific catalog #137119B

"Large orifice" pipet tips:

- These are p20/p200 yellow or clear pipet tips with the tip cut off so that the *Arabidopsis* seeds don't get stuck inside. These can be ordered directly from Fisher Scientific (catalog # 211972B) or can be made by cutting off the ends of pipet tips with a razor blade.

REDEExtract-N-Amp Plant PCR Kit:

- One kit (Sigma catalog # XNA-P) contains 12 ml of Extraction Buffer, 12 ml of Dilution Buffer, and 1.2 ml of PCR Reaction Mix. The kit can be stored for long periods of time at -20°C.

PCR Primers:

- The following primers were ordered from Integrated DNA Technologies (www.idtdna.com):
Primer # 1501: 5' - CTC CTG GAT GGA GTA ATG GTG - 3'
Primer # 2221: 5' - AAG CTC GAT ACC AGC AGC TTG - 3'

These primers flank a 784 bp region of *CRYPTOCHROME1* that contains the 452 bp deletion in the *cry1* mutants.

25 nmole quantities of each were ordered, and resuspended in 250 ml of water to obtain a concentration of 100 pmoles/ ml. This was aliquotted into 10 ml aliquots, and stored at -20°C until the week before the lab. For the lab, a 10 ml aliquot was thawed and diluted with 90 ml of water to obtain a concentration of 10 pmoles/ ml. This is the concentration that is used by the students in this laboratory exercise.

Appendix D: Alternative Procedure for Extracting Plant DNA

1. Remove a single *Arabidopsis* leaf (or entire seedling) with forceps. Place in a 1.5-ml microfuge tube.
2. Use a small, plastic pestle (fits into bottom of tube) to grind the leaf, without added buffer, for 15 seconds.
3. Add 400 μ l of Extraction Buffer (200 mM Tris-HCl pH 7.5-8, 250 mM NaCl, 25 mM EDTA, 0.5% SDS). Vortex 5 seconds. (At this stage, samples can be held at room temperature for up to one hour).
4. Centrifuge tube 1 minute at full speed (over 10,000 rpm) to pellet debris.
5. Transfer 300 μ l of the supernatant into a fresh, labeled 1.5-ml microfuge tube.
6. Add 300 μ l isopropanol; mix and leave at room temperature for 2 minutes.
7. Centrifuge the tube for 5 minutes at full speed to pellet the DNA.
8. Remove **all** of the supernatant and dry the pellet by incubating the open tubes at 37°C for several minutes. Do not over-dry the pellet.
9. Add 100 μ l of sterile dH₂O to the pellet and dissolve by gentle shaking.
10. Use 2-4 μ l of resuspended DNA per PCR reaction. Unused DNA should be frozen if needed for future use.

Source of small, plastic pestles (designed for 1.5-ml tubes) is Research Products International Corp., Mount Prospect, IL 60056. (phone: 1-800-323-9814; fax: 1-847-635-1177)

Appendix E: Assessment

The following summarizes the results of student evaluations of this laboratory exercise. 67 students returned the evaluations. Average and standard deviations from an N of 67 are indicated.

Indicate the strength of your agreement or disagreement with the following statements by filling in the appropriate circle (circles labeled 1 through 5). A higher number generally indicates satisfaction. 1 = disagree strongly, 5 = agree strongly

1. This lab helped me to understand how genetic analyses are done: 3.88 +/- 0.85
2. This lab helped me understand genetics: 3.56 +/- 0.85
3. This lab helped me understand complete versus incomplete dominance: 3.89 +/- 2.43
4. As a result of this lab, I now feel confident using a Pipetman: 4.04 +/- 1.10
5. I was already proficient using a Pipetman prior to this lab: 2.91 +/- 1.59
6. This lab helped me understand how PCR works: 3.35 +/- 1.18
7. This lab helped me understand how genotype can be determined: 3.77 +/- 1.15
8. As a result of this lab, I now feel confident performing gel electrophoresis: 3.48 +/- 1.47
9. I was already proficient performing gel electrophoresis prior to this lab: 2.42 +/- 1.82
10. My PCR product was clearly visible on the gel: 3.57 +/- 1.54
11. The lab manual chapter for this lab was easy to follow: 3.48 +/- 1.55
12. I enjoyed this lab: 3.24 +/- 1.77

Student comments:

- interesting lab
- maybe create a less subjective way to determine hypocotyl length
- This one was fun because we got to see the connection between the phenotype and genotype of the plants.
- I thought this lab was interesting, but that has to do with the fact that I like genetics.
- I really enjoyed this lab. It helped me understand how DNA could be identified.
- This lab involved a lot of tedious counting. The sequencing, however, was powerful in demonstrating the relationship between genotype and phenotype.