# Play Mendel Advanced:

# Student Handout – Lab Procedures & Assignments

## PROCEDURE 1 – Plant P generation seeds

**Materials**

| | | |
|---|---|---|
| 4 strains of Arabidopsis seeds | Potting soil | Fertilizer |
| 32 plastic pots per group | 4 solid trays per group | 4 trays with holes per group |
| Cheesecloth or paper towels | Weighing boats | Disposable Pasteur pipettes |
| Labeling tape and marker | Plastic wrap | Watering can |

1. Cut 32 pieces of cheese cloth or paper towel to fit the bottom of a pot. Place one piece in the bottom of each pot. This will prevent soil from escaping during watering.

2. Place potting soil in a container and moisten with water. The moisture level of the soil should resemble a wet sponge. Add fertilizer according to package directions and mix thoroughly. Wear gloves when handling fertilizer and fertilized soil.

3. Fill each pot loosely with soil. Do not compress the soil as you fill the pots.

4. Stack one tray with drainage holes inside a solid tray. Repeat to make four pairs of trays, which will simply be called trays moving forward.

5. Your group will plant two trays for each of the two experiments. Using labeling tape and a permanent marker, label each tray with your group number, date and the name of the experiment (see examples below).

| Group 1 - Date<br>Glabrous Experiment | Group 1 - Date<br>Agamous Experiment |
|---|---|

6. Label eight pots for each strain of Arabidopsis (see examples below).

| Group 1<br>Col-1 | Group 1<br>*gl1-1* | Group 1<br>Ler-0 | Group 1<br>*ag-1* |
|---|---|---|---|

7. Fill a weighing dish with water. Working with one seed stock at a time, sprinkle a portion of the seeds of one stock into the water. Mix the seeds in the water by pipetting up and down slowly using a disposable Pasteur pipette. This will help to separate the seeds and make it easier to capture them individually. Be sure to use a different weighing dish and pipette for each seed stock to prevent cross-contamination.

8. Use the pipette to draw up individual seeds and place them on the surface of the soil. Plant nine seeds, evenly spaced, in each pot (Figure 1). Do not cover the seeds with soil.

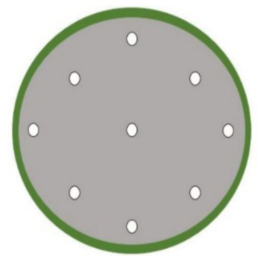


Figure 1. Placement of 9 seeds on soil surface (Price *et al.*, 2018).

9. For the glabrous experiment, place four Col-1 and four *gl1-1* pots in each of the two trays. For the agamous experiment, place four of the Ler-0 and four *ag-1* pots in each of the two trays (Figure 2).

10. Wrap each tray tightly with plastic wrap to maintain moisture levels during germination.

11. Optional – Place all of the trays inside a cold room or refrigerator at 4°C for 2-3 days. This process, known as stratification, mimics winter conditions and promotes uniform germination of the seeds. Skip this step if you do not have access to adequate refrigeration space.

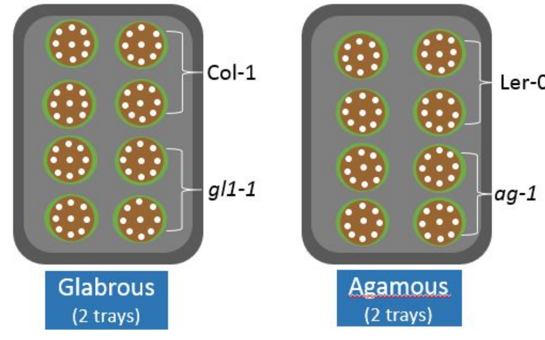



Figure 2. Illustration of placement of pots within trays for the two experiments (Price *et al.*, 2018).

12. Place the trays under fluorescent lights.

13. Remove the plastic wrap once you see seedlings emerge from the soil (approximately 7 days after planting).

14. You will begin watering the plants regularly once the wrap is removed. Do not water directly into the pots. Add water to the tray to a depth of ½ inch once or twice a week. Be careful not to overwater the pots or allow the soil to dry out.

**ASSIGNMENT 1 – Observe growth and record phenotypes**
Complete the following tasks in your lab notebook:

1. Define key terms related to plant growth and anatomy:
   Rosette, inflorescence, silique, stratification, trichome, germination, bolting, and senescence.

2. Make detailed drawings of the plants. Make notes about any visible differences between the four different strains.

3. Describe the Arabidopsis life cycle by noting details about the different growth stages such as how many leaves are present in the rosette, when flowering begins, when siliques form, when senescence begins.

4. Observe the Col-1 and *gl1-1* plants, and the Ler-0 and *ag-1* plants. Identify what trait is different between each pair of plants. Make detailed drawings of the traits with notes to describe the differences. Make a note of when each trait was first noticeable (*e.g.*, in rosette stage or after flowering).

**ASSIGNMENT 2 – Analyze the inheritance of the *ag-1* allele**

1. Complete the worksheet for this assignment.

2. In your lab notebook, define key genetic terms:
   Genotype, phenotype, dominant and recessive.

**PROCEDURE 2 - Perform a genetic cross between *gl1-1* homozygous mutants and the Col-1 reference strain.**

**Materials**
Headband magnifier
Tweezers
Labeling tape and marker

1. Working with a pot of Col-1 plants, find an inflorescence with at least two closed buds with barely visible petals at the tip (Figure 3A).

2. Carefully remove any siliques, partially open buds and flowers from the selected inflorescence (Figure 3 A-D).

3. Using tweezers and a headband magnifier, carefully remove the sepals, petals and stamens from one of the selected buds (Figure 3E-G). This process is very difficult, be careful and take your time. Try not to damage the carpel. If you do, that's okay, just move on to another closed bud and try again.

4. Select a fully open flower on a *gl1-1* plant. Use tweezers to remove the flower from the plant, squeezing at the base of the flower to expose the anthers (Figure 3H).

5. Pollinate the Col-1 flower by brushing the anthers of the *gl1-1* flower over the exposed carpel of the Col-1 flower (Figure 3I). Make sure to transfer some pollen to the stigma during this process.

6. Use tape to label your cross (see example below). When labeling a genetic cross the female parent is always listed first. In this case, the Col-1 plant is the female parent and the *gl1-1* is the male parent. These plants represent the P generation.

| Group 1 - Date |
|---|
| Col-1 x *gl1-1* |

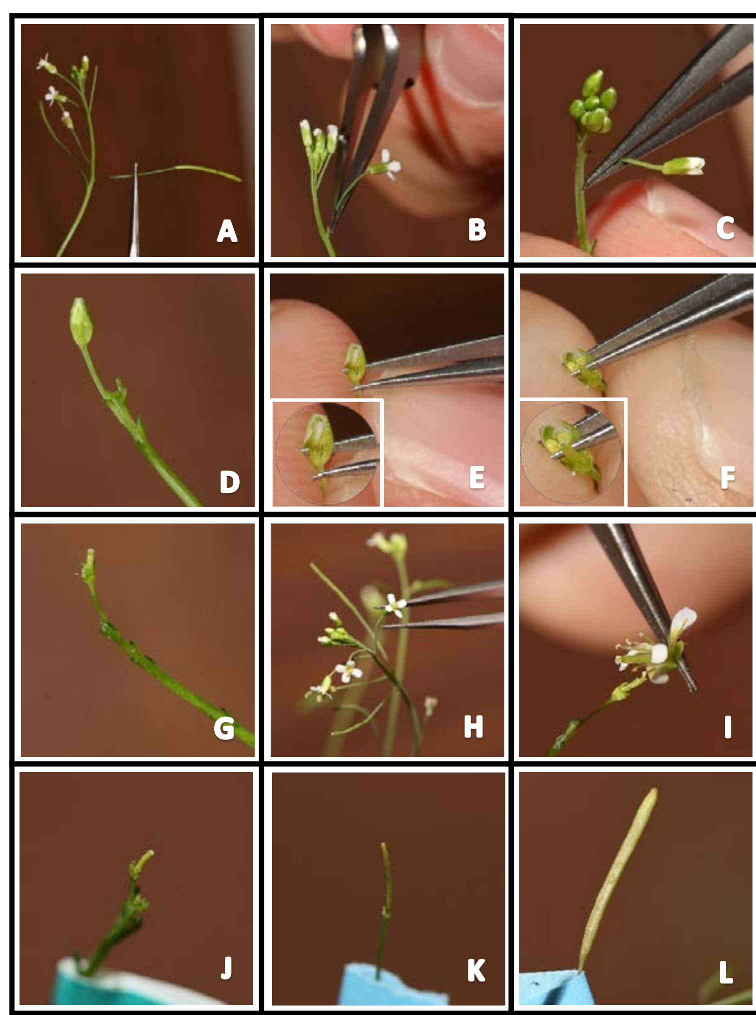



**Figure 3**. Steps involved in performing a genetic cross between two parent plants (Price *et al.*, 2018).

7. Place tape loosely around the inflorescence below the exposed carpel (Figure 3J).

8. Repeat steps 1-7 using *gl1-1* as the female parent plant. Label crosses accordingly (see example below).

| Group 1 - Date |
|---|
| *gl1-1* x Col-1 |

9. Observe the plants daily for signs of a successful cross. Continue to water plants for one week after performing crosses.

10. One week after performing the crosses stop watering the plants. Allow the pot dry out for two weeks.

**ASSIGNMENT 3 – Observe cross outcomes**
Complete the following tasks in your lab notebook:

1. Observe plants daily and make notes on whether or not the crosses were successful.

2. Make drawings that show the results of a successful and unsuccessful cross.

3. Calculate the ratio of successful to unsuccessful crosses for the class.

4. Make a detailed drawing of a flower, showing the location of each of the structures listed below. Define the role of each structure in reproduction.
   Anther, filament, stamen, stigma, style, ovary, ovule, and carpel.

**PROCEDURE 3 – Collect F1 seeds**

**Materials**
Scissors
Eppendorf tubes
Labeling tape and marker

1. Working with the Col-1 x *gl1-1* crosses first, locate all siliques from successful crosses.

2. Label a 2 mL Eppendorf tube with your group number, date, plant number, generation and cross type (see example below). Prepare one tube for each silique to be collected.

| Group 1, date, plant # |
| --- |
| F1: Col-1 x *gl1-1* |

3. Use scissors to carefully remove each silique from the plant and place in a tube (1 silique/tube). Gently tap the tube on a table several times to release the seeds.

4. Repeat steps 1-3 for the *gl1-1* x Col-1 crosses.

5. At this point, the P generation plants can be discarded.

6. Allow the seeds to dry in the Eppendorf tubes for two weeks. This process will reduce the internal moisture content of the seeds leading to higher germination rates.

**PROCEDURE 4 – Plant F1 seeds**

**Materials**

F1 seeds collected in Procedure 3
18 plastic pots per group
Cheesecloth or paper towels
Labeling tape and marker
18 Plastic bouquet sleeves per group
Potting soil
2 solid trays per group
Weighing boats
Plastic wrap
Fertilizer
2 trays with holes per group
Disposable Pasteur pipettes
Watering can

1. Cut 18 pieces of cheese cloth or paper towel to fit the bottom of a pot. Place one piece in the bottom of each pot. This will prevent soil from escaping during watering.

2. Place potting soil in a container and moisten with water. The moisture level of the soil should resemble a wet sponge. Add fertilizer according to package directions and mix thoroughly. Wear gloves when handling fertilizer and fertilized soil.

3. Fill each pot loosely with soil. Do not compress the soil as you fill the pots.

4. Stack one tray with drainage holes inside a solid tray. Repeat to make two pairs of trays.

5. Your group will plant one tray using seeds from the Col-1 x *gl1-1* crosses, and another tray using seeds from the *gl1-1* x Col-1 crosses. Label each tray with your group number, generation and the type of cross (see example below).

| Group 1<br>F1: Col-1 x *gl1-1* | Group 1<br>F1: *gl1-1* x Col-1 |
|---|---|

6. Label eight pots for each of the two types of crosses.

7. Label one pot each for Col-1 and *gl1-1* seeds.

8. Fill a weighing dish with water. Working with one stock at a time, sprinkle a portion of the seeds into the water. Mix the seeds in the water by pipetting up and down slowly using a disposable Pasteur pipette. This will help to separate the seeds and make it easier to capture them individually for planting. Be sure to use a different weighing dish and pipette for each seed stock to prevent cross-contamination.

9. Use the pipette to draw up individual seeds and place them on the surface of the soil. For the Col-1 x *gl1-1* seeds and the *gl1-1* x Col-1 seeds, plant nine seeds evenly spaced in each pot. For the Col-1 and *gl1-1* control pots, place 10-20 seeds per pot. These pots will serve as controls for comparison. Do not cover the seeds with soil.

10. Place the pots in their corresponding trays. The control pots can also be placed in these trays, one in each.

11. Wrap each tray tightly with plastic wrap.

12. Optional – Place all of the trays inside a cold room or refrigerator at 4°C for 2-3 days. This process, known as stratification, mimics winter conditions and promotes uniform germination of the seeds. Skip this step if you do not have access to adequate refrigeration space.

13. Place the trays under fluorescent lights.

14. Remove the plastic wrap once you see seedlings emerge from the soil (approximately 7 days after planting).

15. You will begin watering the plants regularly once the wrap has been removed. Do not water directly into the pots. Add water to the tray to a depth of ½ inch once or twice a week. Be careful not to overwater the pots or allow the soil to dry out

16. Complete Assignment 4 three to four weeks after planting.

17. Vent sixteen plastic sleeves. To vent the sleeves, cut two 1″ x 1″ openings approximately six inches from the bottom on opposite sides of the sleeve.

18. After six weeks of growth, cover the pots with plastic sleeves to avoid cross-contamination.

19. Continue to water plants for one more week, then let plants dry-out for two additional weeks.

**ASSIGNMENT 4 – Observe and record phenotypes**

Complete the following tasks in your lab notebook:

1. Make detailed drawings of the plants.

2. Compare the phenotype of the plants grown from Col-1 x *gl1-1* crosses to the plants grown from *gl1-1* x Col-1 crosses. Are there differences? Why or why not?

3. Define P, F1 and F2 generations.

**PROCEDURE 5 – Collect F2 seeds**
Scissors
Envelopes
Fine metal sieve

1. Carefully cut open and remove the plastic sleeve from the pots.

2. Using scissors, cut the plants free from the soil and roots. Using a new envelope for each plant, place the plant material inside an envelope. Seal the envelope and press it several time to break open the siliques and release the dry seeds.

3. Cut open a corner of the envelope being careful not to lose any plant material. Holding a metal sieve over a clean piece of paper, pour the contents of the envelope into the sieve. Continue to run the material through the sieve until the seeds are free of soil, plant material and other contaminants.

4. Transfer the collected F2 seeds into a labeled Eppendorf tube (see example below). Allow the seeds to dry for two weeks before planting.

| Group 1, date, plant # |
|---|
| F2: Col-1 x *gl1-1* |

5. Repeat steps 1-4 until all plants have been harvested. At this point, the F1 plants can be discarded.

**PROCEDURE 6 – Plant F2 seeds**

**Materials**

| | | |
|---|---|---|
| F2 seeds collected in Procedure 5 | Potting soil | Fertilizer |
| 18 plastic pots per group | 2 solid trays per group | 2 trays with holes per group |
| Cheesecloth or paper towels | Weighing boats | Disposable Pasteur pipettes |
| Labeling tape and marker | Plastic wrap | Watering can |

1. Cut 18 pieces of cheese cloth or paper towel to fit the bottom of a pot. Place one piece in the bottom of each pot. This will prevent soil from escaping during watering.

2. Place potting soil in a container and moisten with water. The moisture level of the soil should resemble a wet sponge. Add fertilizer according to package directions and mix thoroughly. Wear gloves when handling fertilizer and fertilized soil.

3. Fill each pot loosely with soil. Do not compress the soil as you fill the pots.

4. Stack one tray with drainage holes inside a solid tray. Repeat to make two pairs of trays.

5. Your group will plant one tray using seeds from the Col-1 x *gl1-1* crosses, and another tray using seeds from the *gl1-1* x Col-1 crosses. Label each tray with your group number, generation and the type of cross (see example below).

| Group 1<br>F2: Col-1 x *gl1-1* | Group 1<br>F2: *gl1-1* x Col-1 |
|---|---|

6. Label eight pots for each of the two types of crosses.

7. Label one pot each for Col-1 and *gl1-1* seeds to serve as controls.

8. Fill a weighing dish with water. Working with one seed stock at a time, sprinkle a portion of the seeds into the water. Mix the seeds in the water by pipetting up and down slowly using a disposable Pasteur pipette. This will help to separate the seeds and make it easier to capture them individually for planting. Be sure to use a different weighing dish and pipette for each seed stock to prevent cross-contamination.

9. Use the pipette to draw up individual seeds and place them on the surface of the soil. For the Col-1 x *gl1-1* seeds and the *gl1-1* x Col-1 seeds, plant nine seeds evenly spaced in each pot. For the Col-1 and *gl1-1* control pots, place 10-20 seeds per pot. These pots will serve as controls for comparison. Do not cover the seeds with soil.

10. Place the pots in their corresponding trays. The control pots can also be placed in these trays, one in each.

11. Wrap each tray tightly with plastic wrap.

12. Optional – Place all of the trays inside a cold room or refrigerator at 4°C for 2-3 days. This process, known as stratification, mimics winter conditions and promotes uniform germination of the seeds. Skip this step if you do not have access to adequate refrigeration space.

13. Place the trays under fluorescent lights.

14. Remove the plastic wrap once you see seedlings emerge from the soil (approximately 7 days after planting).

15. You will begin watering the plants regularly once the wrap has been removed. Do not water directly into the pots. Add water to the tray to a depth of ½ inch once or twice a week. Be careful not to overwater the pots or allow the soil to dry out.

**ASSIGNMENT 5 – Analyze segregation for F2 generation plants**

1. Make detailed drawings of the plants in your lab notebook.

2. Compare the phenotype of the plants grown from Col-1 x *gl1-1* crosses to the plants grown from *gl1-1* x Col-1 crosses. Are there differences? Why or why not?

3. Complete the worksheet for this assignment.

**ASSIGNMENT 6 – Formulate next-step research questions**
Complete the following tasks in your lab notebook:

1. Think about what you have learned about the *gl1-1* and *ag-1* mutations, the two reference strains, and the life cycle of Arabidopsis. Write about what else you would like to learn about Arabidopsis, and what types of experiments you would like to conduct using this plant.

2. Write a research question for a new experiment using Arabidopsis.

3. Formulate a hypothesis to test based on your research question.

**AGAMOUS WORKSHEET** - Complete this worksheet as part of Assignment 2

**Table 1.** Data sheet to calculate the ratio of plants displaying the reference phenotype versus the mutant phenotype.

| | # Plants with reference phenotype | # Plants with mutant phenotype | Reference to Mutant Plant Ratio |
|---|---|---|---|
| Group 1 | | | |
| Group 2 | | | |
| Groups 1 + 2 | | | |

1. How might combining the data from both groups affect the ratio of reference to mutant plants determined by your group? Explain your reasoning.

Answer the questions below after combining the class data.

2. Did combining the data from both groups cause the ratio to change?

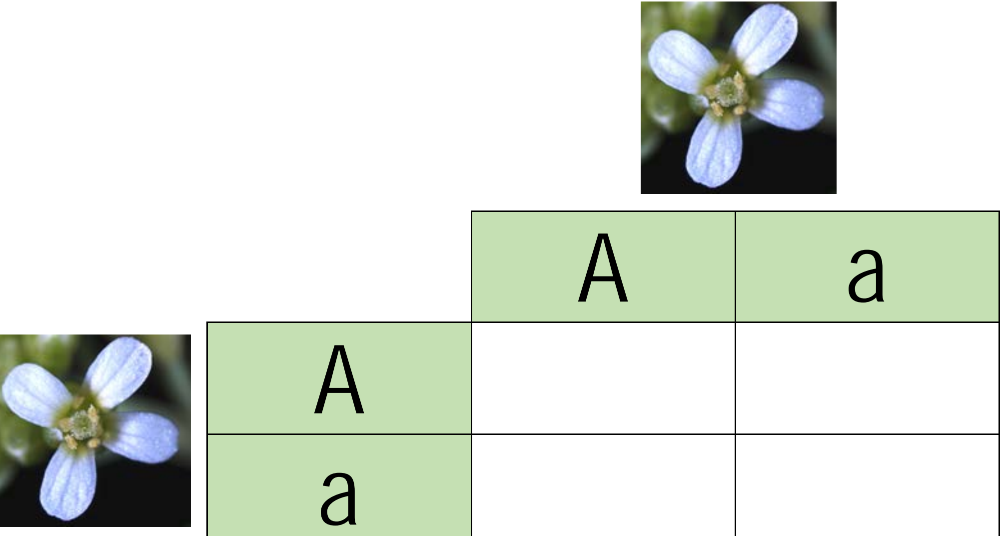
3. If so, explain how and why the ratio changed.

4. Why is this important to the process of science?

5. Based on the ratio obtained by the class, is the *ag-1* allele dominant or recessive?

AGAMOUS WORKSHEET

**Table 2.** Complete the Punnett square below. This will serve as evidence to support your finding from Question 5.



| | A | a |
|---|---|---|
| A | | |
| a | | |

1. Which offspring will display the reference phenotype?

2. Which offspring will display the mutant phenotype?

**GLABROUS WORKSEET** - Complete this worksheet as part of Assignment 5

**Table 1.** Data sheet to calculate the ratio of plants displaying the reference phenotype versus the mutant phenotype.

| | # Plants with reference phenotype | # Plants with mutant phenotype | Reference to Mutant Plant Ratio |
|---|---|---|---|
| Group 1 | | | |
| Group 2 | | | |
| Groups 1 + 2 | | | |

1. How might combining the data from both groups affect the ratio of reference to mutant plants determined by your group? Explain your reasoning.

Answer the questions below after combining the class data.

2. Did combining the data from both groups cause the ratio to change?

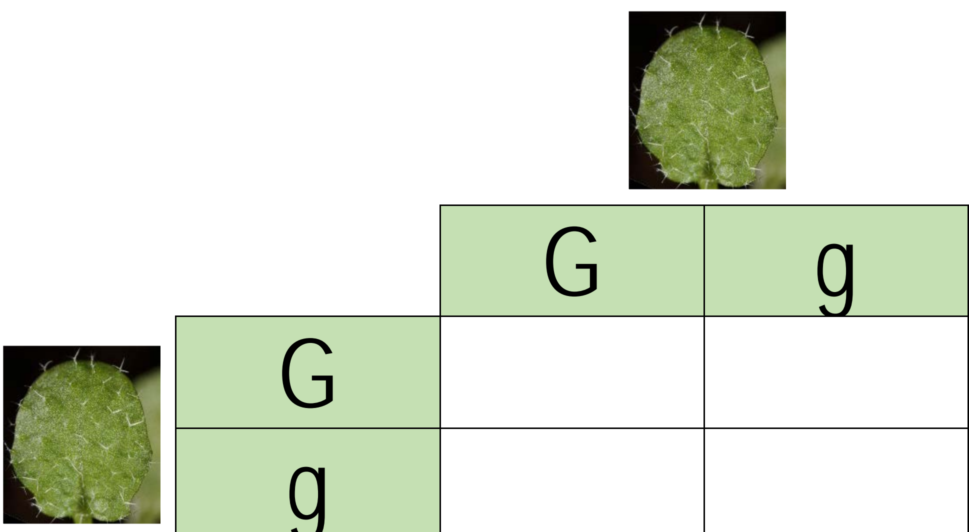
3. If so, explain how and why the ratio changed.

4. Why is this important to the process of science?

5. Based on the ratio obtained by the class, is the *gl1-1* allele dominant or recessive?

## AGAMOUS WORKSHEET

**Table 2.** Complete the Punnett square below. This will serve as evidence to support your finding from Question 5.



| | G | g |
|---|---|---|
| G | | |
| g | | |

1. Which offspring will display the reference phenotype?

2. Which offspring will display the mutant phenotype?