Appendix A

• **Planting Protocols**

  1. Planting in Soil for Classroom Experimentation
  2. Planting in Agar for Classroom Experimentation
  3. Thinning Soil-Grown Plants
  4. Feeding *Arabidopsis*

• **Sterile Environment Directions**

• **Materials List**

• **Recipes**
Planting

As students design and begin their experiments, it is helpful to think about these four major strategies for investigation:

1. students can deprive the plants of something (e.g. nutrients, light, water, etc.)
2. students can expose the plants to something extra (e.g. nutrients, light, water, etc.)
3. students can examine root growth and development
4. students can examine germination (do the seeds sprout?)

Depending on what strategy(ies) your students are using in their investigation, they will want to plant their plants in different ways.

**Strategy #1 and Strategy #2:** If your students want to add or take away something and then examine how the plant grows and develops, they should plant their seeds in soil. Soil is a rich medium where plants normally grow and is easy to manipulate by, for example, adding materials during watering. Planting in soil is the best way to mimic growth in nature.

**Strategy #3:** Planting in soil makes examining root growth and development a little tricky. If your students want to examine roots during their experiment, planting the seeds in agar petri dishes makes the roots visible. Although the plants won’t fully mature normally in a small space like a petri dish, initial root growth and development will be easy to observe and document.

**Strategy #4:** Planting in soil also makes germination (or sprouting) difficult to observe. If your students want to determine if a their treatment affects germination, they can plant their seeds on agar. Then, the seeds and early sprouts will be easily visible.

**NOTE:** Because the agar plates are a nice environment for plants to grow, they are also a nice environment for bacteria and fungi to grow. The other stuff growing is not only unappetizing, it’s bad for the plants. Thus, if your students choose to grow their plants on agar, they will need to sterilize their seeds. The protocol is provided below.

**Planting in Soil**

**Materials**

<table>
<thead>
<tr>
<th>[PREP will provide all consumable materials unless otherwise noted]</th>
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<tbody>
<tr>
<td>• soil</td>
</tr>
<tr>
<td>• trays with clear lids to hold pots</td>
</tr>
<tr>
<td>• pots</td>
</tr>
<tr>
<td>• plant shelves</td>
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<tr>
<td>• small piece of filter paper</td>
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</table>

**Procedure**

1. Fill pots to the brim with soil.
2. Place the pots in a tray.
3. Add about 2 liters of water to the tray (the water will soak into the soil from underneath).
4. Allow to soak 1-2 hours (or up to a day).
5. Measure out about 10-20 seeds onto a piece of filter paper.
6. Sprinkle seeds over the soil.
7. Cover the trays with clear lids and put in a refrigerator for 2-4 days. This process is called vernalization - putting the plants in the cold mimics winter, and taking them out of the cold mimics springtime, the plant’s cue to germinate.
8. Take trays out of the refrigerator and put on plant racks with lights on 16 hours, off 8 hours.
9. Leave lids on (keeps the soil moist) until the first leaves appear.
10. Water the pots when they feel light (weight-wise) and look dry. You will most likely not need to water much until the plants really start to grow.
Planting in Agar

Materials  [PREP will provide all consumable materials unless otherwise noted]

• agar plates
• small piece of filter paper
• seeds (wild-type and mutant)
• micropipet
• 95% ethanol
• sterile toothpicks (optional)

• lights
• plant racks
• seed sterilization fluid in microfuge tubes
• sterile micropipet tips
• Parafilm® or plastic wrap

Procedure
1. Soak seeds in sterilization fluid for 5 minutes, shaking occasionally.
2. Allow seeds to settle and pipet off the liquid.
3. Add 95% ethanol and shake again.
4. Pipet off the liquid and repeat steps 3 and 4.
5. Once you’ve pipetted off the liquid again, carefully pipet seeds onto sterile filter paper.
6. Allow the seeds to dry (about 5-15 minutes).
7. OPTION A: Sprinkle the seeds on the agar and seal the petri dish shut with Parafilm® or plastic wrap.
   OPTION B: Wet a sterile toothpick and touch it to a seed. Carefully stick the seed to the agar surface.
8. Put dishes in a refrigerator for 2-4 days (this is called vernalization).
9. Take dishes out of the refrigerator and put on plant racks with lights on 16 hours and off 8 hours.

Here is a photograph of Arabidopsis growing in an agar petri dish:
Seed Sterilization Fluid

To make 100 ml of seed sterilization fluid, combine:
• 0.1 ml Triton-X detergent
• 30 ml sterile water
Mix gently until completely combined. Then add:
• 70 ml ethanol
Mix gently to combine.

Agar plates for growing Arabidopsis

To make 1 liter of sterile agar for plates, combine the following in a large Erlenmeyer flask:
• 4.3 g MS salts
• 10 g sucrose
• 900 ml distilled water
Use 1.0 M NaOH or KOH to make the solution pH 5.7. Add enough water to make total volume 1 liter. Then add:
• 8 g agar
Autoclave for 20 minutes at 120°C or microwave until the agar is completed dissolved, being careful that the agar doesn’t boil over. Cool the solution to 50°C and add:
• 50 mg kanamycin (to prevent bacterial growth on the plates)
Swirl to mix thoroughly. Pour the agar solution into petri dishes, one liter should make 40-50 plates (10 cm diameter).

Thinning Soil-Grown Plants

Students will most likely plant more plants than will comfortably ‘fit’ into a small pot. Thus, when students can see the cotyledons and hypocotyl above the soil, they should thin the plants. Thinning is, in essence, reducing the number of plants in the pot so that when the plants are adults, they will all fit. Plants that are crowded actually grow and develop differently than plants that have plenty of space. So unless your students want to study the effects of crowding, they should thin their plants. If student have only 8-10 plants growing in a pot, they don’t need to thin them.

Materials
• plants with cotyledons and hypocotyl showing above the soil
• tweezers

Procedure
• You want to have about 8-10 plants left when you’re done
• You want the plants that are left to be spread out around the pot as much as possible, so remove a plant if it is really close to another plant
• DON’T MOVE ANY OF THE PLANTS THAT ARE LEFT - moving them can kill them if you’re not careful
• Now, carefully pluck out the extra plants with a pair of tweezers until you have only 8-10 plants left
• If you’d like, you can examine and make observations about the plants you remove
Feeding Arabidopsis

Although Arabidopsis can grow without the addition of any extra nutrients, scientists usually water with a water/nutrient solution called Hoagland’s Solution. You can plant the seeds in water-soaked soil and, once the true leaves have sprouting, continue to water with regular water or water with Hoagland’s. PREP will provide Hoagland’s solution for you, but the recipe is included here in case your students want to know appropriate levels of nitrogen, potassium, and other nutrients to use in designing their experiments.

Hoagland’s Solution for Happy Arabidopsis Plants

<table>
<thead>
<tr>
<th>Component</th>
<th>ml/50L</th>
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<tbody>
<tr>
<td>2M KNO₃</td>
<td>125</td>
</tr>
<tr>
<td>2M Ca(NO₃)₂</td>
<td>125</td>
</tr>
<tr>
<td>*Iron (Fe)</td>
<td>75</td>
</tr>
<tr>
<td>2M MgSO₄</td>
<td>50</td>
</tr>
<tr>
<td>1M NH₄NO₃</td>
<td>50</td>
</tr>
<tr>
<td>**Minors</td>
<td>50</td>
</tr>
<tr>
<td>1M KH₂PO₄</td>
<td>25</td>
</tr>
<tr>
<td>pH to 5.3 with 10% H₂SO₄</td>
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*Iron:
- Fe 138 10.04
- Na EDTA 7.74

Note: Can order from CIBA-GEIGY (sprint 138 15g/L)
Iron chelate micro nutrient with no need to add Na EDTA.

**Minors:
- H₃BO₃ 2.86
- MnCl₂ x 4 H₂O 1.81
- ZnSO₄ x 7 H₂O 0.22
- CuSO₄ 0.051
- H₃MoO₄ x H₂O 0.09
- OR (Na₂MoO₄ x 2 H₂O) 0.12

NOTE: PREP can provide any of the materials if your students need them for their investigations.

REFERENCES


STERILE ENVIRONMENT

Purpose
The purpose of the sterile environment box is to provide inexpensive conditions where sterile work can be conducted.

Background Information
*Arabidopsis* can be grown on agar plates so students can observe root growth and hypocotyl extension. Agar plates are not only ideal for growing plants, they are ideal for growing bacteria and mold as well. To minimize contamination when growing plants, agar plates should be poured in a sterile environment. Sterilized seeds should also be plated on the agar plates in a sterile environment. Sterile hoods may not be readily available; however, a sterile environment can be constructed by completing the protocol below. Having several of these inexpensive sterile environments will allow several students to work at the same time.

Note: Boxes can be assembled by the teacher or by students. If completed by students, this can become an activity on aseptic techniques in the laboratory.

Materials
- one medium size cardboard box (minimum L18” x W18” x H12” or 0.5 m x 0.5 m x 0.33 m),
- tape
- clear plastic bag
- scissors or box cutter
- 95% ethanol in a spray bottle.

Procedure
1. Remove the top and one side of the box using scissors or a box cutter. *If the box is not square, remove the top and one of the long sides.* (Caution students on the use of a box cutter.)

Note: It is important to think about how the box needs to be arranged to accomplish your tasks. The top of the box will become the window that you
use to view your work and the open end will become your access to the inside of the box.

2. Place the clear plastic bag inside the box orienting the opening in the plastic bag with the open end of the box.

3. Tape the plastic bag to the sides of the box, securing the the top of the bag across the opening in the top of the box. The opening in the bag should be allowed to hang freely over the open end of the box. Trimming off any excess may be necessary.

4. Cut two slits in the open end of the bag, creating slots for your hands to access the sterile environment.

5. Once construction is complete, spray the inside of the sterile environment (the inside of the plastic bag) with 95% ethanol.

**Aseptic Techniques Protocol**

1. Spray your hands with 95% ethanol before putting them into the sterile bag environment.

2. Dip forceps and other necessary instruments into 95% ethanol before placing them into the sterile bag environment.

3. Spray the outside of any other objects that enter the sterile environment of the plastic bag.

**Note:** Anytime something is removed from the sterile environment of the plastic bag (including your hands), it must be resterilized before it is placed back inside the plastic bag.

**Follow-up**

Challenge your students to think about the following questions:
- Why is it important to resterilize the inside of the box before each use?
- Why is it important to sterilize everything, including your hands, before they enter the box?

Students can complete an investigation, swabbing agar plates using sterile and non sterile procedures in order to see what grows. **For health purposes, it is important that students do not open plates that have unknown organisms growing on them.**

**TEACHER NOTES**

Resterilize the inside of the box (plastic bag) before each use.

Agar dishes right out of the package should be sterile and need not be sprayed with ethanol.

Students can research the history of sterilization in hospitals and the problems that occurred prior to sterilization.
Appendix B

• Classical genetics [Mendel]

• Molecular genetics [DNA]
Appendix C

• Additional rounds of research

• Additional resources

• Additional activities
Appendix D

• Glossary

• Websites and Other Resources
Glossary

**Anther:** The anther is located at the top of the stamen, the plant’s male reproductive organ, and is the site where pollen is stored.

**Average:** An average is one way to represent a group of numbers. To find an average, add all of the values together and divide this total by the number of values you added.

**Bolt:** The bolt, or the stem, is the plant structure that supports the leaves and flowers and transports water and nutrients throughout the plant.

**Cauline leaves:** The cauline leaves, located on the bolt, are the second set of leaves to grow on an *Arabidopsis* plant.

**Cotyledon:** The cotyledons are a food source in the seed until the plant grows enough to perform photosynthesis. They are embryonic leaves and are one of the first plant structures to be visible above the soil.

**Ecotype:** An ecotype is like a strain or breed. For example, all dogs are in the same genus and species (*Canis familiaris*), but can be different breeds or strains (e.g. Cocker spaniel, German Shepherd, Poodle, etc.). In *Arabidopsis*, strains are called ecotypes.

**Filament:** The filament is the stem-like part of the stamen, the plant’s male reproductive organ.

**Flower:** A flower is a specialized reproductive structure in a plant. Flowers can contain either male or female parts (incomplete flowers) or both (complete flowers).

**Gene:** A gene is a segment of DNA that codes for a protein (or, technically, a chain of amino acids called a polypeptide).

**Genome:** A genome is all of the DNA in an organism.

**Germination:** Germination is when a seed sprouts to start forming a plant.

**Hypocotyl:** The hypocotyl is the structure of a plant between the roots and the cotyledons.

**Kinase:** A kinase is a protein, in this case an enzyme, that adds phosphate (PO$_3^-$) groups to other proteins. A kinases reverses the action of a phosphatase.

**Mean:** (see ‘Average’) The mean is the average. To find the mean, add a group of numbers together and divide that total value by the number of numbers in the group.

**Median:** The median is the middle value in an ordered set of numbers.

**Mode:** The mode is the most frequently occurring number in a set of numbers.

**Model organism:** A model organism is an organism that is used commonly in scientific research because it is easy to study and manipulate. Often, model organisms are small (easy to house), have short generation times (no waiting), and have lots of progeny (many babies to study). For example, *Arabidopsis* is a model plant and *Drosophila*, or the fruit fly, is a model animal.

**Mutant:** A mutant is an organism that is different from a ‘normal’ or wild-type version of that organism because of a mutation, or change, in its DNA.
**Mutation:** A mutation is a change in the sequence of a piece of DNA. The change can be small (a single nucleotide of DNA) or large (several thousand nucleotides), and may or may not alter an organism’s phenotype, or characteristics.

**Ovary:** The ovary is a structure in a plant (or animal) that stores the organism’s eggs, or ovules. In *Arabidopsis*, it’s called a gynoecium.

**Ovule:** An ovule in an egg, the female reproductive cell.

**Petal:** Petals are modified leaves surrounding the reproductive organs. They are often brightly colored to attract pollinators.

**Phosphatase:** A phosphatase is a protein, in this case an enzyme, that removes phosphate (PO₃⁻) groups from other proteins. A phosphatase reverses the action of a kinase.

**Phosphorylation:** Phosphorylation, performed by kinases, is the process of adding a phosphate (PO₃⁻) group to a protein.

**Pistil:** The pistil is the plant’s female reproductive organ, which comprises three parts: the stigma, the style, and the ovary.

**Pollen:** Pollen is the sperm of plants.

**Protein:** A protein is a chain of amino acids. Proteins are coded for by an organism’s DNA and perform almost every function in an organism’s body.

**Root:** Roots are plant structures that grow below ground and help plants stay stable in weather, absorb water and minerals, and store food.

**Rosette leaves:** The rosette leaves, located at the base of the plant, are the first set of ‘true’ leaves (cotyledons the first leaf-like structures to grow above ground, but are not true leaves) to grow on an *Arabidopsis* plant.

**Seed:** A seed contains a baby plant and the baby plant’s food supply, wrapped in a seed coat.

**Seed pod:** In *Arabidopsis* plants, the seed pod is a green bean-shaped structure that stores and protects the seeds as they develop.

**Sepal:** Sepals are modified leaves that surround the base of the flower.

**Stamen:** The stamen is a plant’s male reproductive organ, which stores/supports the pollen. The anther is at the top of the stamen and the filament is the stem-like part of the stamen.

**Stem:** The stem, or bolt in *Arabidopsis*, is the plant structure that supports the leaves and flowers and transports water and nutrients throughout the plant.

**Stigma:** The stigma is farthest protruding part of the plant’s female reproductive organ (the pistil), the site where pollen is supposed to land.

**Stoma (plural is stomata):** Stomata are tiny pores on the leaves of plants that open and close for gas exchange and release of water.
**Strain:** A strain is like a breed. For example, all dogs are in the same genus and species (*Canis familiaris*), but can be different breeds or strains (e.g. Cocker spaniel, German Shepherd, Poodle, etc.). In *Arabidopsis*, strains are called ecotypes.

**Style:** The style is the stem-like part of the plant’s female reproductive organ. The stigma is at the top of the style.

**Thinning:** In *Arabidopsis*, thinning is the process by which a gardener removes plant sprouts to ensure that, as the plants grow, each will have sufficient space to develop.

**Transpiration:** Transpiration is the process by which plants lose water from their leaves.

**Vernalization:** In *Arabidopsis*, vernalization is a process through which scientists mimic springtime for their plants. The newly planted seeds are put into a refrigerator for 2-4 days (like winter), and then removed and placed in good lighting (like spring). Planting with vernalization triggers more seeds to germinate than planting without vernalization.

**Wild type:** A wild-type organism is one that is considered ‘normal’, like organisms found in the ‘wild’. A mutant is any organism whose DNA is different from the wild-type’s DNA. Mostly, a wild type is used as a standard for comparison, not because it is the ‘best version’ of an organism. Scientists define one or several strains of an organism to be the wild type, so everyone is comparing to the same standard across the world.
Websites and Other Resources

For more information about plant genomics

• Plant Sciences Strategic Steering Committee: http://ceprap.ucdavis.edu/plantscience/gen.htm

For more information on the National Science Foundation’s Plant Genomics Research Program

• Plant Genome Research: http://www.nsf.gov/bio/dbi/dbi_pgr.htm

For more information on the Functional Genomics of Plant Phosphorylation project (PlantsP)

• The PlantsP website: http://plantsp.sdsc.edu/

For more information about human genomics

• Human Genome Project Information: http://www.ornl.gov/hgmis/
• Human Genome Research: http://www.science.doe.gov/ober/hug_top.html

For more general information about Arabidopsis thaliana

• The Arabidopsis Information Resource (TAIR): http://www.arabidopsis.org/
• Links to Arabidopsis web resources: http://www.arabidopsis.org/links/

For more information about Arabidopsis phenotypes

• The Nottingham Arabidopsis Stock Centre: http://nasc.nott.ac.uk/
  Go to ‘Search catalogue’, then ‘Browse’, then ‘Picture Book’ for over 600 images of biochemical, color, flower, form, and hormone mutants
Contact information for scientists studying plant genomics

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