### LESSON B2–2: Asexual Propagation

<table>
<thead>
<tr>
<th>Instructional Time</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Time</td>
<td>4 days followed by several months of post-propagation care</td>
</tr>
</tbody>
</table>
| NGSS Performance Expectations | HS-LS1-2  
|                     | HS-LS1-3  
|                     | HS-LS1-4  |
| Common Core Standards | CC.11-12.R.ST.1  
|                     | CC.11-12.R.ST.8  
|                     | CC.11-12.R.ST.10  
|                     | CC.11-12.SL.4  
|                     | CC.11-12.SL.5  
|                     | CC.11-12.L.6  |
| National AFNR Standards | PS.03.01.03.a  
|                     | PS.03.01.03.b  
|                     | PS.03.01.03.c  
|                     | PS.03.01.04.a  
|                     | PS.03.01.04.b  
|                     | PS.03.01.04.c  |
| Essential Question | How are different techniques used to propagate plants asexually? |
| Student Learning Objectives | At the conclusion of this lesson, students will be able to:  
|                     | 1. Explain physiological processes that take place during adventitious root formation.  
|                     | 2. Propagate plants using different techniques, and assess the results.  
|                     | 3. Describe how media are prepared for tissue culture.  
|                     | 4. Perform tissue culture techniques under aseptic conditions. |
ENGAGING Activities: Teacher

(Opening activity or interest approach—access prior learning / stimulate interest / generate questions)

1. Ask the students how they feel about cloning animals, including people. The question might produce some strong opinions. Ask how they would feel about cloning plants. Then, tell them that people have been cloning plants for thousands of years. Hand out the Engaging Activity “Cloning: Pros and Cons” provided with this lesson plan. The activity asks students to assign pro or con labels to the statements provided. Give the students a few minutes to complete the activity, and then discuss their answers.

ANSWER KEY for “Cloning: Pros and Cons” Activity

1. Cloning helps in producing offspring of the same species quickly and in large quantities. PRO
2. Cloning reduces genetic diversity, which could lead to greater susceptibility of crops to diseases and environmental changes. CON
3. Cloning makes it easier to predict the time between planting and harvesting, providing the advantages of crop cultivation and earning for farmers. PRO
4. Cloning interferes with the natural evolution of plants, leading to an imbalance in the natural way of crop growth. CON
5. Cloning done on an extensive scale can result in food production becoming more commercialized, adversely affecting Third World countries. CON
6. Cloning results in plants with better tolerance to pesticides and chemical fertilizers. PRO
7. Cloning is less expensive than sexual propagation of plants. PRO
8. Cloning allows the reproduction a single plant with excellent nutritional benefits, resulting in similar plants with the same benefits. PRO
9. Cloning produces plants that are identical in looks, and in landscaping, this can result in a monotonous appearance. CON
10. Cloning results in quicker yields, and therefore food problem issues can be reduced. PRO

EXPLORING Activities: Teacher

(Lesson description; materials needed / probing or clarifying questions; students think, plan, and investigate and then organize collected information; rubrics)

2. Display an apple, such as a Honeycrisp apple, and ask the students how thousands of identical apple trees can be produced for growth in orchards given the fact that apple trees grown from seed rarely produce fruit that is true to the variety.

1. Ask the students to come up with questions that must be answered to be successful with asexual propagation. List the questions provided for all to see. Lead a discussion during which the students group similar questions and prioritize the questions in terms of what ones need to be answered first. Keep this list readily available, and refer to it as the lesson proceeds.

2. Assign the reading of the corresponding E-unit.
Have the students practice various asexual propagation techniques using different plant types. Have them take stem cuttings of woody and herbaceous plants. Have them take leaf cuttings of certain house plants. Have them separate and divide perennials. Have them graft fruit trees. Have them perform tissue culture techniques. Have them evaluate the results. Which techniques were successful? Which failed?

Have the students design a lab to expand their understanding of plant propagation. Suggested research topics include:

- Impacts of different rooting hormone concentrations on rooting success
- Age of cuttings and rooting success
- Hormone concentration effects with tissue culture
- Bottom heat, no bottom heat; mist system, no mist system

Tissue culture kits are available for purchase online. One vendor is Carolina Biological Supply Company: http://www.carolina.com/living-organisms/plant-tissue-culture-and-plant-physiology/plant-tissue-kits/10610.ct?

Conduct lab activities provided in this lesson: (1) “Tissue Culture of Blackberry Seeds,” (2) “Tissue Culture of Venus Flytraps,” and (3) “Tissue Culture of Boston Ferns.”

TEACHER NOTES for Lab Activities

Helpful Hints:

- Prior to starting the experiment, autoclave (sterilize) a brown paper bag sealed with masking tape that contains the equipment needed for the lab. You could have student assistants do this or have the students in the class do this. Also, prepare sterile distilled water and 10 percent bleach solution in small jars, such as baby food jars.
- Blackberry seeds require scarification and stratification to germinate. Seeds may not germinate if they dry out before stratification.
- The labs for tissue culture of Venus flytraps and tissue culture of Boston ferns work well immediately following the lab for aseptic culture of blackberry seeds.
- Contamination by fungus or bacteria will be evident in a short time if microorganisms were introduced to the medium. These microorganisms thrive on the nutrient rich agar medium and, if present, will grow swiftly to cover the medium. Contaminated test tubes have no value other than showing what contaminated test tubes look like. Wash and save the tubes for the next experiment.

Anticipated Findings:

Lab Activity 1

The cut blackberry seeds will germinate in about 7 to 10 days. The uncut seeds will not grow because of the impervious seed coat.

The blackberry seedlings can be lifted from the medium, planted in small pots, and grown into whole plants using procedures different from those used for seedlings.
Lab Activity 2

The Venus flytraps will continue to proliferate on the multiplying medium, and within a month or two, you will have 100 or more plants in your multiplying medium culture tubes.

The Venus flytraps in the rooting medium will begin to produce roots.

The Venus flytraps in peat moss will continue to grow. High humidity found in a terrarium provides good growing conditions. A 2-liter soft-drink bottle will work fine for this project.

Lab Activity 3

The Boston ferns will continue to proliferate on the multiplying medium, and within a month or two, you will have a hundred or more plants in your cultures.

The Boston ferns in the rooting medium will begin to produce roots.

The Boston ferns in peat moss will continue to grow in the 2-liter soft-drink bottle. Later, transplant it to a pot.

Ideas for Additional Experiments:

Once you have achieved success with tissue culture techniques, try propagating other plants by tissue culture. Some plants commonly propagated by tissue culture include hosta, African violet, orchid, tobacco, and rose. Conduct research first to determine the requirements for the media to be used for the species selected.

Assess the completed lab reports for (1) “Tissue Culture of Blackberry Seed,” (2) “Tissue Culture of Venus Flytrap,” and (3) “Tissue Culture of Boston Ferns” using the “Lab Report Scoring Rubric” provided, or modify the scoring rubric for your situation.
# Lab Report Scoring Rubric

<table>
<thead>
<tr>
<th>Possible Points</th>
<th>Sections</th>
<th>Section Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Title:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ The title is concise and appropriate.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Introduction:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ The research problem is defined.</td>
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<tr>
<td></td>
<td>✓ The objectives and purpose of the lab are clearly explained.</td>
<td></td>
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<tr>
<td></td>
<td>✓ The hypothesis is stated and is logical.</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Procedures or Methods:</td>
<td></td>
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<tr>
<td></td>
<td>✓ The procedure of the lab is stated with sufficient clarity to allow its replication.</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Findings or Results:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ Data generated from the research is well presented.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ Tables, graphs, or similar summary formats are used effectively.</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Conclusions:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ The meanings of the results are clearly explained and are supported by the data collected.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ Conclusions closely parallel the hypothesis that was initially stated for the study.</td>
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<tr>
<td>10</td>
<td>Recommendations:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ Suggestions are provided that would implement the research findings and/or further the research on the topic.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>References:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ Citations and references adhere to the proper format.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Presentation:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ Spelling and grammar are correct.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ The overall appearance is attractive.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ The report is clear and concise.</td>
<td></td>
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<tr>
<td></td>
<td>✓ The report reflects thoughtful scientific inquiry.</td>
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</tr>
<tr>
<td><strong>Total Score</strong></td>
<td><strong>F</strong></td>
<td><strong>D</strong></td>
</tr>
<tr>
<td><strong>0–59</strong></td>
<td><strong>60–69</strong></td>
<td><strong>70–79</strong></td>
</tr>
</tbody>
</table>
EXPLAINING Activities: Teacher

(Concepts explained and vocabulary defined; students analyze their exploration)

1 Key Terms Defined:

- **adventitious root**: a root that arises from any plant part other than by the normal development of seedling roots or their branches
- **agar**: a solidifying agent consisting of a polysaccharide obtained from seaweeds
- **asexual propagation**: the reproduction of new plants using only the vegetative parts of the parent plant
- **callus**: a mass of unorganized parenchyma cells that forms on a wounded surface
- **dedifferentiation**: the process by which previously developed differentiated cells near the vascular tissues become meristematic tissue
- **endogenous root**: a type of adventitious root that forms within the stem; root initiation is usually near vascular bundles and near the source of hormones, carbohydrates, etc.
- **exogenous root**: a type of adventitious root initiated within callus that forms at the site of a wound or, in the case of a cutting, where the cut was made
- **explants**: small pieces of plant material carefully removed from the parent plant
- **meristematic tissue**: plant tissue that contains undifferentiated cells from which new cells form and is located in plant zones where growth occurs
- **meristemoid**: a small, triangular stomatal precursor cell that functions temporarily as an undifferentiated stem cell in a meristem
- **plantlets**: small plants or plant shoots that form during tissue culture and are capable of developing into complete plants
- **subculture**: a group of cultured cells or tissue transferred to fresh medium during the tissue culture process
- **tissue culture**: the practice of growing cells or small pieces of plant tissue on artificial media under sterile conditions
- **totipotency**: the potential of a cell to differentiate into any type of cell depending on the special function required

2 Have the students write a research report for the labs completed.

3 Have the students deliver an oral report to the class on the results of their lab work. Use of multimedia is encouraged.

4 Lead a class discussion to identify patterns related to the tissue culture labs conducted by the students.

5 Return to the list of questions the students created at the beginning of the lesson. Be sure all have been answered satisfactorily.
Using the “Descriptions of Asexual Propagation Techniques” worksheet provided with this lesson, have the students provide a brief description of common asexual propagation techniques.

### ANSWER KEY for “Descriptions of Asexual Propagation Techniques” Activity

<table>
<thead>
<tr>
<th>Asexual Propagation Technique</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cutting</td>
<td>A cutting, generally 2 to 5 inches in length, consisting of the stem and leaf portions of a plant</td>
</tr>
<tr>
<td>Leaf cutting</td>
<td>A cutting consisting of an entire leaf or a portion of a leaf</td>
</tr>
<tr>
<td>Leaf-petiole cutting</td>
<td>A cutting consisting of the leaf blade and the petiole</td>
</tr>
<tr>
<td>Leaf-bud cutting</td>
<td>A cutting consisting of the leaf blade, the petiole, a bud at the base of the petiole, and a portion of the stem</td>
</tr>
<tr>
<td>Grafting</td>
<td>The process in which the stem of one plant is made to grow on the roots of another plant</td>
</tr>
<tr>
<td>Budding</td>
<td>A form of grafting in which the scion consists of a single bud</td>
</tr>
<tr>
<td>Layering</td>
<td>A method of asexual reproduction in which roots form on a stem while the stem is still attached to the parent plant</td>
</tr>
<tr>
<td>Separation</td>
<td>The process in which vegetative plant structures are removed intact from a plant and planted</td>
</tr>
<tr>
<td>Division</td>
<td>The process in which the plant roots or the entire plant is cut into sections to make two or more plants from the original plant</td>
</tr>
</tbody>
</table>

### ELABORATING Activities: Teacher


2. Arrange a field trip to a tissue culture laboratory or an enterprise that is involved in asexual propagation practices. Instruct the students to be prepared to ask questions.

3. Have the students inspect perennial plants in their yards or in the school land lab for the need to be divided. If the plants appear overgrown and crowded and if they have not been divided for a number of years, help the students propagate them by using division techniques discussed in this lesson.

4. Have the students select an herbaceous plant and a woody plant for asexual propagation. Allow them to choose the method of propagation and to evaluate the results using the “Asexual Propagation Applications” form provided.
Assign the “Checking Your Knowledge” questions from the related E-unit, and grade the answers.

### ANSWER KEY for Asexual Propagation: “Checking Your Knowledge” Questions

<table>
<thead>
<tr>
<th>Matching</th>
<th>Multiple Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. g</td>
<td>1. c</td>
</tr>
<tr>
<td>2. b</td>
<td>2. c</td>
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<td>3. c</td>
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<td>9. a</td>
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<td>10. d</td>
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</table>

### Short Answer

1. A leaf cutting is a cutting consisting of an entire leaf or a portion of a leaf.
2. A stem cutting is a cutting, generally 2 to 5 inches in length, consisting of the stem and leaf portions of a plant.
3. Separation is the process in which vegetative plant structures are removed intact from a plant and planted.
4. Budding is a form of grafting in which the scion consists of a single bud.
5. Tissue culture is the practice of growing cells or small pieces of plant tissue on artificial media under sterile conditions.

Have the students present an oral report that demonstrates their mastery and understanding of the development of adventitious roots. A poster and PowerPoint presentation are suitable visual aids. Assess the presentation using the “Assessing Adventitious Root Formation Presentation” scoring rubric. Provide the rubric to the students in advance, and base the scoring on the material found in the corresponding E-unit.

Administer a written test to determine mastery of terms and concepts. A sample test has been provided in the “Asexual Propagation: Assessing What You’ve Learned” activity.

### ANSWER KEY for “Asexual Propagation: Assessing What You’ve Learned” Activity

1. **How do plant hormones influence adventitious root formation?**

   Adventitious root initiation is promoted by high levels of auxins and low levels of cytokinins. Low levels of auxins and high levels of cytokinins promote adventitious bud/shoot development.
2. **How do adventitious roots develop?**

   *Phase 1:* Dedifferentiation, or remeristemation, of parenchyma cells takes place. Dedifferentiation is the process by which previously developed differentiated cells near the vascular tissues become meristematic tissue.

   *Phase 2:* The root initiation phase is characterized by root initials consisting of meristematic cells that divide and form slightly organized cell groups.

   *Phase 3:* The development of meristemoids marks the transition from the root initiation phase to the root primordium formation phase. During the root primordium formation phase, the first well-developed young root meristems became visible.

   *Phase 4:* Cells of the root primordia elongate. Vascular tissues form and connect the root to the cutting.

3. **Why is an intermittent mist system used in asexual plant propagation?**

   The mist is applied at regular intervals between dawn and sunset to reduce water loss through transpiration. Misting is continued until the roots form and can absorb moisture for the plant. Keeping humidity very high reduces stress on the plants.

4. **How are media prepared for tissue culture?**

   Typically, media preparation involves preparation of stock solutions in the range of 10x to 100x concentrations using high-purity chemicals and demineralized water.

   Most plant tissue culture media are commercially prepared and made available as dry powders. The dry powders are dissolved in distilled or demineralized water. Sugar, organic supplements, and agar are added, the pH is adjusted, and the media are diluted to a final volume (usually 1 liter). The basic steps are:

   1. Measure out approximately 80 percent of the final required volume of tissue culture grade water (e.g., 800 ml for a final volume of 1,000 ml). Use a container twice the size of the final volume.
   2. While stirring the water, add the powdered medium, and stir until completely dissolved.
   3. Rinse the original container with a small amount of demineralized water to remove traces of the powder, and add the water to the solution made in step 2.
   4. Add desired heat-stable supplements (e.g., sucrose, gelling agent, vitamins, etc.).
   5. Add what remains of the 200 ml of demineralized water to bring the medium to the final volume.
   6. While stirring, adjust the pH of the medium to the desired level by adding NaOH, HCl, or KOH.
   7. If a gelling agent is used, heat until the solution is clear.
   8. Dispense the medium into the culture vessels.
   9. Sterilize the medium in an autoclave at 121°C and 15 psi for 20 minutes or for the time described under protocols for the specific medium.
   10. Filter-sterilize and add hormones and other heat-sensitive organic compounds to the medium after autoclaving.
   11. Allow the medium to cool before using.
5. **How is tissue culture performed?**

   In the first stage, explants are carefully removed from the parent plant. The explants are cleaned and placed on sterile agar medium in glass containers.

   In the second stage, the cells of the explants multiply in one of two ways. The cells may form callus, which differentiates and develops into small plantlets consisting of leaves and stems. The other possibility in stage two involves the rapid multiplication of plantlets. Multiplication of explants is accomplished by placing cytokinins in the medium.

   The third stage involves the formation of roots. When the plantlets have developed and there is a desire for them to generate roots, they are separated and transplanted to another medium that contains higher levels of auxins to promote root formation. The plantlets are also given higher light intensity in preparation for stage four.

   In the fourth stage, the plantlets are prepared for normal growing conditions. They are removed from glass containers. The growing medium is gently washed from the plant roots to reduce the growth of potentially harmful bacteria or fungi. The plantlets are divided, planted in a sterile growing medium, and placed in a greenhouse. A common practice is to place the young plants under an intermittent misting system.
Cloning: Pros and Cons

Read the following statements regarding cloning of plants. Indicate whether you believe each statement should be regarded as an advantage or disadvantage by writing “PRO” or “CON” after the statement.

1. Cloning helps in producing offspring of the same species quickly and in large quantities.
2. Cloning reduces genetic diversity, which could lead to greater susceptibility of crops to diseases and environmental changes.
3. Cloning makes it easier to predict the time between planting and harvesting, providing the advantages of crop cultivation and earning for farmers.
4. Cloning interferes with the natural evolution of plants, leading to an imbalance in the natural way of crop growth.
5. Cloning done on an extensive scale can result in food production becoming more commercialized, adversely affecting Third World countries.
6. Cloning results in plants with better tolerance to pesticides and chemical fertilizers.
7. Cloning is less expensive than sexual propagation of plants.
8. Cloning allows the reproduction a single plant with excellent nutritional benefits, resulting in similar plants with the same benefits.
9. Cloning produces plants that are identical in looks, and in landscaping, this can result in a monotonous appearance.
10. Cloning results in quicker yields, and therefore food problem issues can be reduced.
Tissue Culture of Blackberry Seeds

PURPOSE

The purpose of this activity is to perform tissue culture techniques on blackberry seeds.

OBJECTIVE

Practice tissue culture techniques with blackberry seeds.

MATERIALS

- 1 bamboo food skewer, 8 to 10 inches long, that can be used to manipulate seeds
- 1 small jar of 10 percent bleach solution
- 1 small jar with 100 ml of sterile distilled water
- 1 small jar with sterile potato dextrose agar medium
- 12" × 12" cheesecloth square
- 2 or 3 sterile paper towels
- 4" × 4" cheesecloth square
- 70 percent ethanol
- blackberries
- brown lunch bag
- clear plastic bag (unused) large enough to insert both hands and the materials into (Floral bouquet bags work well.)
- masking tape
- forceps
- razor blade

PROCEDURE

1. Place the skewer, forceps, paper towels, and razor blade in the brown paper bag. Fold the opening, and tape securely. Also, tape the seams of the bag. Autoclave the bag.

2. Clean up the work area, remove all books, and make the area comfortable to work in.

3. Carefully flatten your blackberry between the sterile paper towels.

4. Pick out 10 big, healthy-looking seeds. (Avoid the small or flat seeds; these will not germinate.) Wipe the pulp off on the paper towels.
5. Put the seeds in the center of the smaller cheesecloth square, and lightly fold it over so the seeds will not fall out.

6. Put the seeds and the cheesecloth into the jar of bleach for 10 minutes. A longer period may kill some of the seeds.

7. Roll up your sleeves, and remove any jewelry. Wash your hands with soap up to your elbows. Do not dry your hands, and avoid touching doorknobs.

8. Spray or wipe your hands, arms, and work surface with ethanol for sterilization. Spray or wipe the jar holding the seeds with ethanol, and put it inside the plastic bag.

9. Any time you remove your hands from the bag, wipe your hands and arms with ethanol before reaching back inside the bag.

10. Get the jar of sterile distilled water, wipe it with the larger cheesecloth soaked with ethanol, and put it in the plastic bag.

11. Wipe the brown bag with the cheesecloth soaked in 70 percent ethanol. Place the end of that bag just inside the clear plastic bag. Tear the end of the brown bag, and slide the contents into the plastic bag.

12. After 10 minutes, remove the cheesecloth pouch from the bleach solution with your forceps, and place it into the jar of sterile water.

13. Leave the seeds in sterile water for five minutes (the bleach can kill the seeds if it is not washed away).

14. After five minutes, take out the cheesecloth pouch with your forceps, and open it very carefully. Avoid touching the seeds with your fingers.

15. Take the sterile razor blade, and cut three blackberry seeds in half. Hold the seeds with the forceps (tweezers). Do not touch the seeds with your fingers.

16. Put the six seed halves on the potato dextrose agar medium in a jar using the forceps and/or the bamboo skewer.

17. Take another five seeds, and place them whole on the medium as a control.

18. Record your observations.

19. Prepare a lab report.
Tissue Culture of Venus Flytraps

PURPOSE

The purpose of this activity is to propagate Venus flytraps by tissue culture.

OBJECTIVE

Propagate Venus flytraps using tissue culture techniques.

MATERIALS

- 1 stainless steel needle-like tool to tease the plants apart, such as a stainless steel turkey trussing pin
- 12" × 12" cheesecloth square
- 2 bamboo food skewers, 8 to 10 inches long, that can be used to extract plants from the test tubes and carry plant parts down and into the tissue culture medium
- 2 or 3 sterile paper towels
- 2 test tubes with Venus flytrap multiplying medium
- 2 test tubes with Venus flytrap rooting medium
- 2-liter bottle
- 70 percent ethanol
- brown lunch bag
- clear plastic bag (unused) large enough to insert both hands and the materials into (Floral bouquet bags work well.)
- masking tape
- marker
- parafilm strips
- sphagnum moss
- stage 2 Venus flytraps in multiplying media

PROCEDURE

1. Place the skewers, trussing pin, and paper towels in the brown paper bag. Fold the opening, and tape securely. Also, tape the seams of the bag. Autoclave the bag.
2. Clean up the work area, remove all books, and make the area comfortable to work in.
3. Roll up your sleeves, and remove any jewelry. Wash your hands with soap up to your elbows. Do not dry your hands, and avoid touching doorknobs.

4. Wipe your work station with the cheesecloth soaked in 70 percent ethanol.

5. Wipe your hands and lower arms with the cheesecloth soaked in 70 percent ethanol.

6. Carefully open your plastic bag. Do not breathe into it. All work will be conducted in the bag to reduce contamination.

7. Any time you take your hands out of the bag, wipe them with the ethanol-soaked cheesecloth before returning them to the inside of the bag.

8. Wipe your test tubes with 70 percent ethanol, and put them inside the plastic bag.

9. Wipe the brown bag with the cheesecloth soaked in 70 percent ethanol. Place the end of that bag just inside the clear plastic bag. Tear the end of the brown bag, and slide the contents into the plastic bag.

10. Open a culture tube containing Venus flytraps; use your bamboo skewers to lift the bunch of plants from the tube, and lay it on a piece of sterile paper towel (from inside the paper bag). Do not touch the plants with your fingers!

11. Use the pointed stainless steel tool and the skewers to separate the Venus flytraps into individual plants or small clumps.

12. Put small flytrap clumps into your two multiplying-medium tubes (one small clump each) and into your two rooting-medium tubes. Save three or four plants for potting.

13. Remove culture tubes from the bag, label them with a marker, and seal them with parafilm.

14. Cut the bottom off a clear 2-liter bottle. Fill the bottom with moistened sphagnum peat moss.

15. Put your extra plants in the moist sphagnum peat moss. Rinse any agar from the plants before placing them in the sphagnum. Fungi and bacteria will grow quickly in the agar and damage the flytraps. Then, place the top portion of the 2-liter plastic bottle over and around the bottom, creating a sealed environment.

16. Place the test tubes and the 2-liter bottle in a warm (78-degree) setting. Provide indirect light, 100 foot-candles. Venus flytraps respond well to 16 hours of light under fluorescent fixtures.

17. Record your observations.

18. Prepare a lab report.
Tissue Culture of Boston Ferns

PURPOSE

The purpose of this activity is to propagate Boston ferns by tissue culture.

OBJECTIVE

Apply tissue culture techniques to propagate Boston ferns.

MATERIALS

- 1 stainless steel needle-like tool to tease the plants apart, such as a stainless steel turkey trussing pin
- 12" × 12" cheesecloth square
- 2 bamboo food skewers, 8 to 10 inches long, that can be used to extract plants from the test tubes and carry plant parts down and into the tissue culture medium
- 2-liter bottle
- 2 or 3 sterile paper towels
- 70 percent ethanol
- brown lunch bag
- clear plastic bag (unused) large enough to insert both hands and the materials into (Floral bouquet bags work well.)
- forceps
- jar with prepared multiplying medium for Boston fern
- masking tape
- marker
- parafilm strips
- razor blade
- sphagnum moss
- stage 2 Boston ferns
- test tubes with prepared rooting medium for Boston fern
PROCEDURE

1. Place the skewers, trussing pin, forceps, razor blade, and paper towels in the brown paper bag. Fold the opening, and tape securely. Also, tape the seams of the bag. Autoclave the bag.

2. Clean up the work area, remove all books, and make the area comfortable to work in.

3. Roll up your sleeves, and remove any jewelry. Wash your hands with soap up to your elbows. Do not dry your hands, and avoid touching doorknobs.

4. Wipe your work station with the cheesecloth soaked in 70 percent ethanol.

5. Wipe your hands and lower arms with the cheesecloth soaked in 70 percent ethanol.

6. Carefully open your plastic bag. Do not breathe into it. All work will be conducted in the bag to reduce contamination.

7. Any time you take your hands out of the bag, wipe them with the ethanol-soaked cheesecloth before returning them to the inside of the bag.

8. Wipe your test tubes and jar with 70 percent ethanol, and put them inside the plastic bag.

9. Wipe the brown bag with the cheesecloth soaked in 70 percent ethanol. Place the end of that bag just inside the clear plastic bag. Tear the end of the brown bag, and slide the contents into the plastic bag.

10. Open a culture tube containing Boston ferns; use your forceps or bamboo skewers to lift the bunch of plants from the tube, and lay it on a piece of sterile paper towel (from inside the paper bag). Do not touch the plants with your fingers!

11. Use the pointed stainless steel tool and razor blade to separate the Boston ferns into individual plants or small clumps.

12. Put some Boston ferns (small clumps) into your jar with multiplying medium, and put others into tubes with rooting medium. Save three or four plants for potting.

13. Seal the culture tubes with parafilm and put the lid on the jar, remove them from the bag, and label them with the marker.

14. Place the cultures in a warm (78-degree) setting. Provide indirect light under fluorescent fixtures for 16 hours a day.

15. Cut the bottom off a clear 2-liter bottle. Fill the bottom with moistened sphagnum peat moss.

16. Take your extra plants, and put them in the moist sphagnum peat moss. Rinse any agar from the plants before placing them in the sphagnum. Fungi and bacteria will grow quickly in the agar and damage the ferns. Then, place the top portion of the 2-liter plastic bottle over and around the bottom creating a sealed environment.

17. Put the bottle in your plastic bag, and take it home.

18. Record your observations.

19. Prepare a lab report.
Descriptions of Asexual Propagation Techniques

Provide a brief description of the common asexual propagation techniques.

<table>
<thead>
<tr>
<th>Asexual Propagation Technique</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cutting</td>
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<tr>
<td>Leaf cutting</td>
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<tr>
<td>Leaf-petiole cutting</td>
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<tr>
<td>Leaf-bud cutting</td>
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<tr>
<td>Grafting</td>
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<td>Budding</td>
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<td>Layering</td>
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<tr>
<td>Separation</td>
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<tr>
<td>Division</td>
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</tbody>
</table>
Asexual Propagation Applications

1. Herbaceous plant selected: _________________________
   What propagation technique was used? Why?
   Were rooting hormones used? Which?
   What environmental conditions were provided?
   Did roots form at an endogenous and exogenous site?
   Were your efforts successful? Why or why not?

2. Woody plant selected: _________________________
   What propagation technique was used? Why?
   Were rooting hormones used? Which?
   What environmental conditions were provided?
   Did roots form at an endogenous and exogenous site?
   Were your efforts successful? Why or why not?
# Assessing Adventitious Root Formation Presentation

## SCORING RUBRIC FOR ADVENTITIOUS ROOT FORMATION PRESENTATION

<table>
<thead>
<tr>
<th></th>
<th>Excellent</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Define adventitious root.</td>
<td>Everything is explained in great detail.</td>
<td>Explanation is good but lacks details needed for full understanding.</td>
<td>Explanation lacks clarity or essential information.</td>
<td>No explanation provided.</td>
</tr>
<tr>
<td>Explain the impact of hormones on adventitious root formation.</td>
<td>Everything is explained in great detail.</td>
<td>Explanation is good but lacks details needed for full understanding.</td>
<td>Explanation lacks clarity or essential information.</td>
<td>No explanation provided.</td>
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<tr>
<td>Describe dedifferentiation or remeristemation.</td>
<td>Everything is explained in great detail.</td>
<td>Explanation is good but lacks details needed for full understanding.</td>
<td>Explanation lacks clarity or essential information.</td>
<td>No explanation provided.</td>
</tr>
<tr>
<td>Explain the root initiation phase of adventitious root formation.</td>
<td>Everything is explained in great detail.</td>
<td>Explanation is good but lacks details needed for full understanding.</td>
<td>Explanation lacks clarity or essential information.</td>
<td>No explanation provided.</td>
</tr>
<tr>
<td>Explain the function of meristemoids.</td>
<td>Everything is explained in great detail.</td>
<td>Explanation is good but lacks details needed for full understanding.</td>
<td>Explanation lacks clarity or essential information.</td>
<td>No explanation provided.</td>
</tr>
<tr>
<td>Describe the processes in the final phase of adventitious root formation.</td>
<td>Everything is explained in great detail.</td>
<td>Explanation is good but lacks details needed for full understanding.</td>
<td>Explanation lacks clarity or essential information.</td>
<td>No explanation provided.</td>
</tr>
<tr>
<td>Differentiate between endogenous and exogenous sites of root formation.</td>
<td>Everything is explained in great detail.</td>
<td>Explanation is good but lacks details needed for full understanding.</td>
<td>Explanation lacks clarity or essential information.</td>
<td>No explanation provided.</td>
</tr>
<tr>
<td>Identify environmental conditions that promote adventitious root formation.</td>
<td>Everything is explained in great detail.</td>
<td>Explanation is good but lacks details needed for full understanding.</td>
<td>Explanation lacks clarity or essential information.</td>
<td>No explanation provided.</td>
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</tbody>
</table>
Asexual Propagation: Assessing What You’ve Learned

ESSAY QUESTIONS

Instructions: Provide a detailed explanation of the processes or principles in answering the following questions.

1. How do plant hormones influence adventitious root formation?
2. How do adventitious roots develop?
3. Why is an intermittent mist system used in asexual plant propagation?
4. How are media prepared for tissue culture?
5. How is tissue culture performed?