### LESSON C2–2: Tissue Culture

**Instructional Time**
6 days

**Laboratory Time**
4 days

**NGSS Performance Expectations**
- HS-LS1-2
- HS-LS1-3
- HS-LS1-4

**Common Core State Standard**
- CCSS.ELA-Literacy.RST.11-12.2

**National AFNR Standards**
- PS.03.01.04.a
- PS.03.01.04.b
- PS.03.01.04.c

**Essential Question**
How are plants propagated by means of tissue culture?

**Student Learning Objectives**
Instruction in this lesson should result in students achieving the following objectives:
1. Define tissue culture and its advantages.
2. Explain sterile technique used in plant tissue culture.
3. Describe culture media used in plant tissue culture.
4. Explain the stages of the plant tissue culture process.

**ENGAGING Activities: Teacher**

1. Bring an African violet plant to class. Discuss with the students how a commercial grower of violets would fill an order for 10,000 plants identical to the plant in the classroom. What methods could be used to produce this number of plants? What problems or challenges for the grower (growing space, labor) would this present? Discuss how tissue culture can play a part in solving this propagation problem.
Assign the “What Is Happening?” activity. Show the students the short video clips linked here or clips you may have found. Give the students about a minute to jot down what has happened in each video. Discuss their responses.

- **Video #1**
  “Kenaf Callus Sub-culture—Time Lapse”
  https://www.youtube.com/watch?v=C4xONNBP6Vs

- **Video #2**
  “Cauliflower Tissue Culture—Shoot Organogenesis”
  https://www.youtube.com/watch?v=jHt88X3rS4w

- **Video #3**
  Tissue Culture Mold Contamination—Time Lapse
  https://www.youtube.com/watch?v=_KdAg4us1WQ

**ANSWER KEY for “What Is Happening?”**

1. **Video #1**
   Callus cells are forming in vitro.

2. **Video #2**
   Shoots are forming on a cauliflower explant.

3. **Video #3**
   Fungus has contaminated a culture and is overwhelming the explants.

**EXPLORING Activities: Teacher**

1. Ask the students to come up with questions that must be answered to demonstrate mastery of tissue culture. 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.

3. Arrange a field trip to a facility in which tissue culture is practiced.


5. Conduct one or more of the lab activities provided in this lesson: (1) “Tissue Culture of Apple Seeds” (2) “Tissue Culture of Blackberry Seeds,” (3) “Tissue Culture of Venus Flytrap,” and (4) “Tissue Culture of Boston Fern.”
Helpful Hints:

- Students will understand the process of tissue culture by conducting one or more of the four labs included in this lesson. Kits purchased through science supply companies also work well.
- When using any of the four labs, make sure that all implements are autoclaved and a floral bouquet bag is used.
- The key to making a tissue culture lab successful is to have a sterile environment.
- Prior to starting the experiment, autoclave (sterilize) a brown-paper lunch bag sealed with masking tape that contains the equipment needed for the lab. You could have either student assistants or the students in the class do this. Also, prepare sterile distilled water and 10% 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

- Apple seeds contain compounds that inhibit germination until the seeds are stored in the cold. This mechanism prevents seeds from sprouting in early autumn and being killed by the winter cold. The dormancy of seeds is broken by the cold, and seeds germinate in the spring.
- If the apple used in this experiment has not been exposed to sufficient cold temperatures to break dormancy, then the seeds with coats will not germinate, while the seeds without coats should germinate in a few days.
- If the apple was cold stored for more than a few weeks, then both the seeds with coats and those without coats should germinate at about the same rate.

Lab Activity 2

- The cut blackberry seeds will germinate in about 7 to 10 days. The uncut seeds will not grow because of their impervious seed coats.
- After the blackberry seeds have grown into seedlings, they can be lifted from the medium, rinsed, and planted in small pots. Review Stage 4 of the “Tissue Culture Procedures” presented in the E-unit.

Lab Activity 3

- The Venus flytraps will continue to proliferate on the multiplying medium and, within a month or two, will have produced 100 or more plants in the multiplying-medium culture tubes.
• The Venus flytraps in the rooting medium will begin to produce roots.
• The Venus flytraps in sphagnum 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 4**

• The Boston ferns will continue to proliferate on the multiplying medium and, within a month or two, will have produced a hundred or more plants in the cultures.
• The Boston ferns in the rooting medium will begin to produce roots.
• The Boston ferns in sphagnum moss will continue to grow in the 2-liter soft-drink bottle. Later, they can be transplanted to pots.

Assess the completed lab reports for (1) “Tissue Culture of Apple Seeds” (2) “Tissue Culture of Blackberry Seeds,” (3) “Tissue Culture of Venus Flytrap,” and (4) “Tissue Culture of Boston Fern” using the “Lab Report Scoring Rubric” provided, or modify the scoring rubric for your situation.
<table>
<thead>
<tr>
<th>Possible Points</th>
<th>Section</th>
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<td>5</td>
<td>Title:</td>
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<td>✓ The title is concise and appropriate.</td>
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<td>✓ The objectives and purpose of the lab are clearly explained.</td>
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<td>✓ The hypothesis is stated and is logical.</td>
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<td>Procedures or Methods:</td>
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<td>✓ The procedure of the lab is stated with sufficient clarity to allow its replication.</td>
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<td>Findings or Results:</td>
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<td>✓ Data generated from the research is well presented.</td>
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<td>✓ Tables, graphs, or similar summary formats are used effectively.</td>
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<td>Conclusions:</td>
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<td>✓ The meanings of the results are clearly explained and are supported by the data collected.</td>
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<td>✓ Conclusions closely parallel the hypothesis that was initially stated for the study.</td>
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<td>Recommendations:</td>
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<td>✓ Suggestions are provided that would implement the research findings and/or further the research on the topic.</td>
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<td>✓ Citations and references adhere to the proper format.</td>
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EXPLAINING Activities: Teacher (Concepts explained and vocabulary defined; students analyze their exploration)

1 Key Terms Defined:
   - **agar**: a solidifying agent consisting of a polysaccharide obtained from seaweeds
   - **autoclaving**: a sterilization process that involves the heating of the materials to 245°F for 15 minutes to kill all microorganisms
   - **explant**: living tissue transferred from an organism to an artificial medium for culture
   - **in vitro**: in glass
   - **morphogenesis**: the differentiation and growth of the structure of an organism
   - **plantlet**: a small plant or plant shoot that forms during tissue culture and is capable of developing into a complete plant
   - **sterile technique**: the maintenance of an environment that is free of microorganisms
   - **systemic disease**: a disease that affects the greater part of a plant
   - **tissue culture**: the practice of growing cells or small pieces of plant tissue on artificial media under sterile conditions

2 Have the students write a research report for each lab completed.

3 Have the students deliver oral reports to the class on the results of their lab work. Encourage the use of multimedia technology.

4 Return to the list of questions the students created at the beginning of the lesson. Be sure all have been answered satisfactorily.

5 Assign the “Tissue Culture Stages” activity.

ANSWER KEY for “Tissue Culture Stages”

<table>
<thead>
<tr>
<th>Stage</th>
<th>Processes That Occur</th>
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</table>
| Stage 1   | • Explants are carefully removed from actively growing parts of the parent plant.  
            • Explants are cleaned in a 10% bleach solution, followed by a rinse in sterile water.  
            • Explants are placed on sterile agar medium in glass containers. |
| Stage 2   | • Cells may form callus, a mass of dividing nonspecialized cells.  
            • Cytokinins cause callus cells to differentiate and develop into small plantlets.  
            • Cytokinins encourage adventitious growth, which is seen as an increase in the number of buds (usually six to eight per shoot) on the explants.  
            • Technicians divide clumps of new plantlets and transfer them to new containers with fresh medium to continue the multiplication process. |
<table>
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<tr>
<th>Stage</th>
<th>Processes That Occur</th>
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| Stage 3 | • Plantlets are separated and transplanted to another medium that contains higher levels of auxins to promote root formation.  
• The plantlets are given higher light intensity in preparation for stage 4. |
| Stage 4 | • Plantlets are removed from glass containers.  
• The growing medium is gently washed from the plant roots.  
• The plantlets are divided, planted in a sterile growing medium, and placed in a greenhouse.  
• Young plants are placed under an intermittent misting system and given higher light intensity. |

**ELABORATING Activities: Teacher**

*Applications and extensions; give students the opportunity to expand and solidify their understanding of the concept and/or apply it to real-world situations*

1. Assign the reading of a related Agricultural Career Profile ([http://www.mycерт.com/career-profiles](http://www.mycерт.com/career-profiles)).

2. Assign the “Hormones and Morphogenesis” activity.

**ANSWER KEY for “Hormones and Morphogenesis”**

1. **How would a tissue culture technician promote root formation of plant tissues?**
   
The technician would culture plant tissue on a culture with high auxin:cytokinin ratio.

2. **How would a tissue culture technician promote shoot formation of plant tissues?**
   
The technician would culture plant tissue on a culture with low auxin:cytokinin ratio.

3. **How would a tissue culture technician promote callus formation?**
   
The technician would culture plant tissue on a culture with intermediate levels of auxin and cytokinin.

4. **What happens when only a small amount of cytokinin is in the medium?**
   
No growth will occur.

**EVALUATING Activities: Teacher**

*Summative assessment: scoring tools*

1. Assign the “Checking Your Knowledge” questions from the related E-unit, and grade the answers.
ANSWER KEY for “Checking Your Knowledge”

<table>
<thead>
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<th>Part One: Matching</th>
<th>Part Two: Multiple Choice</th>
<th>Part Three: True/False</th>
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2 Administer a written test to determine mastery of terms and concepts. A sample test has been provided in the “Assessing What You’ve Learned” activity.

ANSWER KEY for “Assessing What You’ve Learned”

1. List five advantages of tissue culture over other means of asexual propagation.
   - Tissue culture techniques allow for much larger numbers of plants to be produced from a single plant in a relatively small space and in a short period.
   - Viruses and other systemic diseases can be eliminated by propagating the quickly dividing cells of the shoot tip.
   - Tissue culture enables growers to produce plants with identical genetic material.
   - Horticultural cultivars can be improved by selecting plants that vary slightly from the parent plant.
   - Tissue culture results in excellent basal branching of some plants.

2. How are media, tools, and bottles or jars sterilized for use in plant tissue culture?
   Materials are autoclaved at 245°F for 15 minutes to kill all microorganisms. Tools are commonly placed in isopropyl alcohol between uses.

3. How can technicians reduce the chance of contaminating cultures?
   Technicians scrub their arms and hands before work to reduce the chance of contaminating cultures. They may also wear face masks, gloves, and hairnets.

4. What are the primary components of tissue culture media?
   - inorganic nutrients (micronutrients and macronutrients)
   - organic supplements (vitamins, amino acids, organic acids, organic extracts, activated charcoal, and antibiotics)
   - carbon and energy sources (sucrose)
   - growth regulators (auxins, cytokinins, gibberellins, and abscisic acid)
5. **How do cytokinins and auxins influence plant tissue cultures?**
   - Cytokinins encourage adventitious growth, which is seen as an increase in the number of buds (usually six to eight per shoot) on the explants.
   - Auxins promote root formation on plantlets.
What Is Happening?

Instructions: Watch the three video clips shown by your teacher, and explain what is happening in each.

1. Video #1

2. Video #2

3. Video #3
Tissue Culture of Apple Seeds

PURPOSE

The purpose of this activity is to perform tissue culture techniques using apple seed embryos.

OBJECTIVE

Demonstrate tissue culture techniques with apple seed embryos.

MATERIALS

- 10% bleach solution
- 2 petri dishes
- 4-inch pot
- 6 test tubes
- 70% ethanol
- apple
- autoclave or pressure cooker
- beakers
- cheesecloth
- clear plastic bags (Floral bouquet bags work well.)
- distilled water
- grow lights
- lab sheet
- lima bean agar (available from science supply companies)
- marker
- single-edge razor blade or scalpel
- sterile soil
- tissue culture medium containing auxins (available from science supply companies)

PROCEDURE

1. Sterilize all equipment used in this experiment by autoclaving. If an autoclave is not available, this process can be accomplished by using a pressure cooker at the same rate as for cooking meat.
2. Prepare sterile lima bean agar according to directions, and place in test tubes (six are needed).

3. Wash hands thoroughly to the elbows with soap. Then rinse, but do not dry. Swab hands and workplace with 70% ethanol. Any item to go into the clear plastic bag in later steps should be wiped with ethanol.

4. Prepare a 10% bleach solution in a beaker.

5. Extract 6 to 10 seeds from an apple, wrap in cheesecloth, place in the 10% bleach solution, and let soak for five minutes. (A few extra seeds are extracted in case seeds are damaged in step 7.)

6. Rinse the seeds for five minutes in a beaker containing distilled water to wash away the bleach.

7. Scrape the seed coats from three apple seeds using a single-edge razor blade. Be careful not to cut the pointed end (embryo) of a seed.

8. Soak the three scraped seeds in 10% bleach solution for five minutes. Wipe the exterior of the beaker with 70% ethanol. Move the beaker inside a large clear plastic bag. Conduct work inside the plastic bag to reduce contamination by microorganisms.

9. Add distilled water to a sterile beaker under the cover of the plastic bag. Transfer the scraped seeds from the bleach solution to the distilled water. Rinse the seeds in distilled water for five minutes.

10. Continuing work within the plastic bag, place one scraped seed onto sterile lima bean agar in each of three test tubes and one unscraped seed onto the agar in each of the other three test tubes.

11. Label the test tubes “Scraped” and “Unscraped.”

12. Observe the test tubes for five days, and record your observations.

13. Prepare tissue culture medium containing auxins according to directions, and place in a petri dish. The auxins will promote root formation. Sterilize the petri dish and medium.

14. Working inside a clear plastic bag, remove the healthiest-looking shoot from the lima bean agar, and place it in the petri dish with tissue culture medium. The other seeds may be discarded. Place the petri dish under grow lights.

15. After roots are formed, transplant the plant to a 4-inch pot with sterile soil.

16. Put the pot with the transplanted plant into a plastic bag to maintain high humidity. Do not place the plant in the bag in full sunlight as the interior will become too warm.

17. After a week, open the bag to expose the plant to lower humidity.

18. After another week, remove the pot from the bag.

19. Observe the growth of the plant.

20. Prepare a lab report.
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 on blackberry seeds.

MATERIALS

- 2 or 3 paper towels
- 4" × 4" cheesecloth square
- 12" × 12" cheesecloth square
- 70% ethanol
- autoclave
- bamboo food skewer, 8 to 10 inches long, that can be used to manipulate seeds
- blackberry
- brown-paper lunch bag
- clear plastic bag (unused) large enough to insert both hands and the materials into (A floral bouquet bag works well.)
- forceps
- lab sheet
- masking tape
- razor blade
- small jar of 10% bleach solution
- small jar with 100 ml of sterile distilled water
- small jar with sterile potato dextrose agar medium
1. Place the skewer, forceps, paper towels, and razor blade in the 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 10% bleach solution 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 70% ethanol for sterilization. Spray or wipe the jar holding the seeds with ethanol, and put the jar inside the plastic bag.

9. Any time you remove your hands from the plastic 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 paper bag with the cheesecloth soaked in ethanol. Place the end of that bag just inside the plastic bag. Tear the end of the paper bag, and slide the contents into the plastic bag.

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

13. Leave the seeds in sterile distilled 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 sterile 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 sterile forceps (tweezers). Do not touch the seeds with your fingers.

16. Using the sterile forceps and/or the sterile bamboo skewer, put the six seed halves on the potato dextrose agar medium in a jar.

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 Flytrap

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
- 70% ethanol
- autoclave
- brown-paper lunch bag
- clear 2-liter bottle
- clear plastic bag (unused) large enough to insert both hands and the materials into (A floral bouquet bag works well.)
- lab sheet
- marker
- masking tape
- parafilm strips
- sphagnum moss
- stage 2 Venus flytraps in multiplying media
1. Place the skewers, trussing pin, and paper towels in the 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% ethanol.

5. Wipe your hands and lower arms with the cheesecloth soaked in 70% 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% ethanol, and put them inside the plastic bag.

9. Wipe the paper bag with the cheesecloth soaked in 70% ethanol. Place the end of that bag just inside the clear plastic bag. Tear the end of the paper 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 a small flytrap clump into each of your two multiplying-medium tubes and into each of 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 moss.

15. Put your extra plants in the moist sphagnum 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°F) 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 Fern

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 or 3 sterile paper towels
- 70% ethanol
- autoclave
- brown-paper lunch bag
- clear 2-liter bottle
- clear plastic bag (unused) large enough to insert both hands and the materials into (A floral bouquet bag works well.)
- forceps
- jar with prepared multiplying medium for Boston fern
- lab sheet
- marker
- masking tape
- parafilm strips
- razor blade
- sphagnum moss
- stage 2 Boston ferns
- test tubes with prepared rooting medium for Boston fern
1. Place the skewers, trussing pin, forceps, razor blade, and paper towels in the 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% ethanol.

5. Wipe your hands and lower arms with the cheesecloth soaked in 70% 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% ethanol, and put them inside the plastic bag.

9. Wipe the paper bag with the cheesecloth soaked in 70% ethanol. Place the end of that bag just inside the clear plastic bag. Tear the end of the paper 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 the jar with multiplying medium, and put others into the 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 the tubes and the jar from the bag, and label them with the marker.

14. Place the cultures in a warm (78°F) 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 moss.

16. Take your extra plants, and put them in the moist sphagnum 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.
# Tissue Culture Stages

*Instructions:* Complete the table by entering the processes that occur in each stage of tissue culture.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Processes That Occur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td></td>
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<tr>
<td>Stage 2</td>
<td></td>
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<tr>
<td>Stage 3</td>
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<tr>
<td>Stage 4</td>
<td></td>
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</tbody>
</table>
Hormones and Morphogenesis

Instructions: Study the diagram, and answer the following questions.

1. How would a tissue culture technician promote root formation of plant tissues?

2. How would a tissue culture technician promote shoot formation of plant tissues?

3. How would a tissue culture technician promote callus formation?

4. What happens when only a small amount of cytokinin is in the medium?
Assessing What You’ve Learned

ESSAY QUESTIONS

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

1. List five advantages of tissue culture over other means of asexual propagation.
2. How are media, tools, and bottles or jars sterilized for use in plant tissue culture?
3. How can technicians reduce the chance of contaminating cultures?
4. What are the primary components of tissue culture media?
5. How do cytokinins and auxins influence plant tissue cultures?