

I. TEACHER NOTES & GUIDELINES

TITLE OF LAB: Plant Cloning

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OVERVIEW OF LAB

DESCRIPTION:

In this lab, students will be using aseptic techniques to clone a plant from a leaf or shoot of a germinating seed. **TEACHER NOTE:** This lab exercise will be conducted over the course of a minimum of 60 days. Plan accordingly.

CURRICULUM CONSIDERATIONS:

This lab will fit into the course of study in the areas of vegetative propagation, asexual reproduction and genetics. It is suitable for Regents, Honors and Advanced Placement Biology.

REAL WORLD APPLICATIONS:

Plant cloning techniques are often used for the mass production of plants. This provides large numbers of genetically identical plants for commercial sales, food production, genetically pure extracts for pharmaceutical purposes as well as other applications.

SAFETY CONSIDERATIONS:

Students will be using denatured alcohol and bleach solutions that are dangerous if ingested, inhaled or spilled. Caution should be taken with the placement of the alcohol lamps in the work area. Fire extinguishers should be available and close at hand. Follow all fire safety procedures.

BACKGROUND INFORMATION

A. SCIENTIFIC VIEWPOINT:

The term plant tissue culture broadly refers to the cultivation *in vitro* of all plant parts (single cells, tissues and organs) under aseptic conditions. In the 1930's Philip R. White demonstrated that tomato roots could be grown indefinitely in a simple nutrient medium containing only sucrose as an energy source, mineral salts essential for plant nutrition, and a few vitamins. Cloning is a type of asexual reproduction in which all offspring are genetically identical to the parent. It can occur naturally (tubers, runners...) or artificially (grafting, cuttings, tissue culture). In modern agriculture and horticulture genetically engineered strains of plants are cloned by sterile techniques similar to those used in this lab.

B. COMMON MISCONCEPTIONS:

Cloning is a relatively new technique.
Plants don't have hormones.
Plants do not react to stimuli.
Plants can only be grown in soil.
Cloning is not a natural process.
Seeds need light to germinate.

OBJECTIVES:

Students may use aseptic techniques to prepare a leaf from a plant for cloning. Once sterile, the students will be able to produce clones of the original plant from pieces of the leaf. The alternate technique uses small seeds such as lettuce.

In the lettuce seed process students will surface sterilize lettuce seeds, aseptically germinate them, and use pieces of the shoots to produce both a callus growth and complete plants. Using the leaf is more prone to mold contamination than the seed technique.

EQUIPMENT/MATERIALS

PROVIDED BY SOTM:

For Both Techniques

- alcohol spritz bottles
- plastic chambers
- Kimwipes
- sterile, distilled water
- 10% bleach spritz bottles
- Tween-20 (detergent)
- 95% ethyl alcohol
- alcohol burners
- parafilm
- tools – scalpel, forceps, spatula, scissors
- media tube racks
- *masks and gloves

Leaf Technique only

- 20% bleach solution
- sterile shoot-root medium plates
- empty sterile petri plates

Seed Technique only

- Sterile Agar Plates
- Basal medium tubes
- Shoot -root medium tubes

PROVIDED LOCALLY:

- 1-100ml beaker (per lab group)
- 1-150ml beaker (per lab group)
- 1-250ml beaker (per lab group)
- matches
- marking pens

Leaf Technique

- Smooth-leaved plants (1 leaf per team)
- large, clear plastic bags with ties
- soil for plants
- flats
- trays to sit beneath flats

Seed Technique

- Nothing extra

ADVANCE PREPARATION:

Swab down the countertops with 10% bleach.

Set up workstations for each lab group with materials needed for lab (refer to list above).

Be sure to have enough healthy, African violet leaves to provide 1 leaf per lab group.

II. PRE-LAB

PRE-LAB EXERCISE TO ELICIT STUDENTS' PRIOR KNOWLEDGE AND MISCONCEPTIONS:

1. Have students create a concept map about what is involved in cloning.
2. Have students in groups develop aseptic lab procedures for cleaning their lab area.
3. Class discussion of growth requirements of plants.
4. What is involved in seed germination?
5. What is a hormone and how does it work? Are they only found in animals?
6. In small groups have students brainstorm the pros and cons of cloning.
7. People can be cloned (MOVIES: *Boys from Brazil*, *Multiplicity*)

DISCUSSION OF PRECONCEPTIONS:

Use the following series of free response questions to elicit a group discussion on tissue culture:

1. What is a clone?
2. Are two parents needed to produce an offspring?
3. What are the advantages to cloning?
4. Are there regions of the plant that are better suited for cloning?
5. What are some ethical concerns revolving around cloning?
6. What are sterile conditions and why are they important?
7. How are they obtained?
8. What is the status of cloning of humans and other mammals?

III. EXPLORATION OF SCIENTIFIC PRINCIPLE & INTRODUCTION OF EXPERIMENTAL PROTOCOL

General Sterile Procedures:

Teach students how to do sterilization by:

- spraying inside of the chamber with alcohol
- wiping with kimwipes
- spraying hands with alcohol
- keeping tools in beaker of alcohol
- flaming tools between each use
- demonstrating the sterile technique within a chamber

PROBLEM: Leaf Technique

1. To prepare a leaf under sterile conditions for tissue culture.
2. To begin a plant tissue culture using sterile leaf.
3. To go from subculture to mature plant to use during inquiry section.

EXPERIMENT AND TECHNICAL OPERATION OF EQUIPMENT:

PART I: LEAF TISSUE CULTURE PROCEDURE

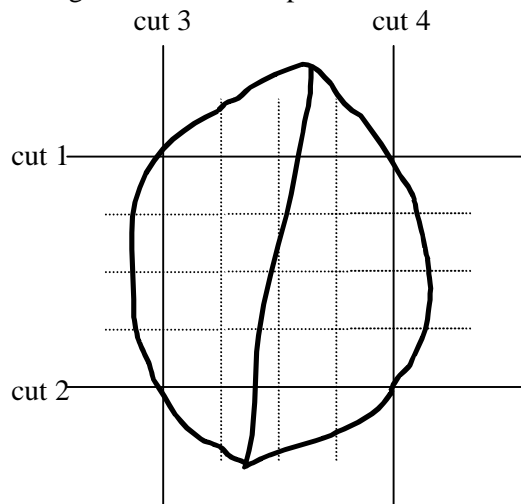
1. Put on facemask, if available

CAUTION: BE SURE THAT THERE ARE NO OPEN FLAMES UNTIL STEP 6 IS COMPLETED.

2. Spray hands with alcohol and allow to air dry. Put on a pair of latex gloves, spray with alcohol and allow to air dry.

3. Spray exterior of work area with alcohol and wipe down. Be sure to spray the table in front of you.
4. Spray interior of chamber with alcohol and wipe down.
5. Place tools in 100ml beaker and fill halfway with ethanol. Spray handles of tools with alcohol and allow to air dry.
6. Spray the following with alcohol, wipe down and place inside sterile chamber:
 - a. 1-150ml beaker
 - b. 1-250ml beaker
 - c. 1 sterile tube of shoot-root medium **NOTE: DO NOT OPEN!!**
 - d. 1 empty petri dish **NOTE: DO NOT OPEN!!**
7. Select a medium size leaf from the African violet plant and cut off close to stem.
8. Rinse leaf under tap water for about 30 seconds.
9. Place leaf in 150ml beaker inside sterile chamber. Pour enough 20% bleach solution to just cover the leaf. Add 2-3 drops of Tween-20 to beaker and swirl to mix. Allow to sit for 10 minutes. Be sure to swirl occasionally.
10. After 10 minutes, pour off bleach/Tween-20 solution into the 250ml beaker inside chamber. Pour enough sterile distilled water into 150ml beaker with leaf to cover leaf and swirl for 1 minute. Pour off water into 250ml beaker. Repeat 2 more times.
11. Light alcohol burner.
12. Remove forceps from alcohol and flame (Hold tool so that the tip is pointing down and away from you. Allow tip to enter flame, alcohol will ignite. Remove tool from flame and allow alcohol to burn off. Allow tool to cool slightly inside chamber before continuing.)
13. Open empty petri dish and use forceps to move leaf from 150ml beaker to the inside of the petri dish.
14. Remove scalpel from alcohol and flame (see step 12).

****Refer to diagram below for steps 15 & 16.****



15. Cut leaf into a square, removing the rounded outer portions (cuts 1-4 on diagram above).
16. Cut what remains of leaf into 1cm squares, refer to dashed lines on diagram above (you should have at least 10 small squares when finished). Replace scalpel in alcohol.
17. Re-flame forceps.
18. Open the sterile medium tube. Use forceps to transfer 5 small squares to the tube. BE SURE that the underside of the leaf

- is in contact with the medium. Use the side of the forceps to gently push leaf squares onto the medium. Cap the tube when completed.
19. Use parafilm to seal edges of the tube before removing from the sterile chamber.
 20. Remove the tube from the sterile chamber and label it with the following information: group name, date, and type of plant.
 21. Store for 60 days. Check weekly for contamination. If plate becomes contaminated see teacher for assistance.

PART II: TRANSPLANTING FIRST SUBCULTURE

1. Once roots and shoots are visible in tissue culture dishes the plants are ready to be transplanted.
2. Prepare work area with: tissue cultures, flats for planting, soil, spatula, large clear plastic bag.
3. Fill flats about 2cm from top with moist soil.
4. Use spatula to remove the medium plug with the young plant. **BE CAREFUL** not to damage any of the roots.
5. Place one young plant in the center of each small pot in your flat.
6. When you have completed transplanting all young plants, place flat in a shallow container of water. **IT IS IMPORTANT TO WATER AFRICAN VIOLETS FROM THE BOTTOM – WATER POURED ON THE LEAVES WILL DAMAGE THE PLANT.**
7. Place bag over tray, flats and seal. **IT IS IMPORTANT THAT FOR THE FIRST 2 WEEKS THE YOUNG PLANTS BE MAINTAINED IN A HUMID ENVIRONMENT.**
8. After the first week the bag can be slowly opened to begin to acclimate the plants to a less humid environment.

PROBLEM: Seed Technique

1. To germinate seeds without contamination.
2. To use pieces of the germinating shoots to grow calluses and new plants.

EXPERIMENT AND TECHNICAL OPERATION OF THE EQUIPMENT

STUDENT ACTIVITY

PART I: SEED GERMINATION PROCEDURE:

(TEACHER CHECKPOINTS ARE NOTED AS <>)*

(3 Students maximum per group)

1. Put on facemask.
2. **CAUTION: BE SURE THAT THERE ARE NO OPEN FLAMES UNTIL STEP 6 IS COMPLETED.**
3. Spray hands with alcohol and allow to air dry. Put on a pair of latex gloves, spray with alcohol and allow to air dry.
4. Spray exterior of work area with alcohol and wipe down. Be sure to spray the table in front of you.
5. Spray interior of chamber with alcohol and wipe down.
6. Place tools in 100ml beaker and fill halfway with 95% ethanol. Spray handles of tools with alcohol and allow to air dry.
7. Spray the following with alcohol, wipe down and place inside sterile chamber:

- a. 1-150ml beaker
- b. 1-250ml beaker
- c. 1 sterile agar plate **NOTE: DO NOT OPEN!!**
8. Do your work inside the chamber as much as possible.
9. Pour seeds into beaker.
10. Pour alcohol (Ethanol) on seeds for 1 minute only. Pour off alcohol carefully. Be careful but prompt with alcohol because it could kill the plant cells if it is left on too long <*>
11. Pour 10% bleach on seeds (15 to 30 minutes). (Alcohol degrades lipids from the surface of seeds. Bleach kills bacteria and fungi on the seeds.)
12. Pour off bleach carefully. You may lose some of the seeds, don't worry! <*>
13. Rinse 3 times with sterile water.
14. Spray the surface of 2 agar plates with alcohol and dry with Kimwipe. Add the plates to your sterile environment.
15. Light Alcohol burners and place on top. (Again, flame tools as needed) Hint: Hold your hands above the flame so that alcohol does not drip on hand ! If your hand is below the flame the alcohol could run onto your hand and ignite!
16. Put 5 seeds per plate on the sterile agar medium. Place seeds beneath the surface of the agar using forceps. <*>
17. Add parafilm around plate to seal.
18. Label plates with name, date and seed type (lettuce, carrot etc.) on the bottom side of each plate.
19. Check your plates three days later for growth and any evidence of contamination.

PART II: CLONING THE SHOOT TISSUE

1. Follow instructions 1 - 6 in the Part I procedures.
2. Surface sterilize the outside of the agar plates with germinating seeds and one tube each of basic growth medium and shoot-root growth medium and place them in the chamber.
3. Spray your hands with alcohol again.
4. **REMEMBER! ALWAYS DIP YOUR SCALPEL, FORCEPS, SCISSORS OR SPATULA INTO THE ALCOHOL AND PASS IT THROUGH THE FLAME TO STERILIZE IT BEFORE EACH PROCEDURE.**
5. Light alcohol lamp and place in a safe place on the top of the aquarium to flame tools.<*>
6. Spray your hands with alcohol again.
7. Working inside the chamber and using sterilized instruments, cut the shoots of a few plants into 1mm long pieces.
8. Using sterilized forceps transfer 4 or 5 pieces of plant to the surface of each tube of medium.<*>
9. Cap each tube with its proper colored cap and remove the tubes from the sterile chamber . Seal the tubes with parafilm.
10. Label each tube with the following information: group name, date, medium,and type of plant.<*>
11. Place the tubes in a well lit area where there will be minimal disturbance. <*>
12. Monitor growth on plates. Note if any contamination occurs.

EVALUATION:

1. Define aseptic techniques
2. Define germination.
3. Why are sterile conditions important?

IV. ELBORATION OF SCIENTIFIC PRINCIPLE: INQUIRY-BASED STUDENT INVESTIGATION

PROBLEM:

Students will develop a long-term experiment using the cloned plants.

HYPOTHESIS OR PREDICTION:

What are the advantages of using genetically identical plants in experimentation rather than plants that may be the same species but are not genetically identical?

EXPERIMENTAL DESIGN:

- If your cloned plants have the same DNA, why might they look different?
- What factors affect plant growth? Why are cloned plants more suitable for this type of experiment?
- How would you measure plant growth?
- Do the rates of photosynthesis vary between cloned and non-cloned plants?
- What materials or environmental conditions would have to be changed to investigate your hypothesis?
- What is your variable? What is your control?
- Given that you have to investigate something about plant growth, make a decision:
 - what to investigate
 - discuss with your teacher
 - set up your experiment

Checkpoint (Teacher checks students' experimental design for feasibility.)

PLAN FOR DATA COLLECTION & ANALYSIS

- How are you going to keep track of your results?
- How can your results be presented visually (table, chart, graph, etc.)?
- How are you going to prove that your data is valuable?
- How are you going to handle inconclusive data?

Checkpoint (Teacher checks students' plan for feasibility.)

CONDUCTING THE EXPERIMENT

Checkpoint (Teacher monitors students' investigations in progress.)

ANALYSIS OF DATA

Checkpoint (Teacher checks students' analysis.)

DISCUSSION OF RESULTS

COMPARE

- Did your data match your hypothesis? Why or why not?
- How does this compare to what you thought when you began this experiment?

PERSUADE

-Prepare to present the results of your investigation to the class. This presentation may take the form of a simulated press conference, a debate, a criminal trial, a board meeting, etc.

RELATE

- Interview local growers. How do the techniques in this lab investigation compare to what they do?
- How does this technique compare to the techniques for cloning animal cells?

V. EVALUATION

POST-LAB SURVEY OF STUDENTS' CONCEPTIONS

1. Have students create a second concept map about what is involved in cloning.
2. Have students return to groups and analyze their aseptic lab procedures for cleaning their lab area. Were their procedures aseptic?
3. Class discussion of growth requirements of plants.
4. Discuss what hormones are and whether or not they are found only in animals.
5. In small groups have students look at the list they brainstormed regarding the pros and cons of cloning. Would they now make any additions or deletions? Why?

TRADITIONAL

1. In theory, will all cloned plants bloom at the same time? Why or why not?
2. Why are aseptic conditions crucial during the preparation of the tissue culture?
3. Name something that had to be added to the agar in order to produce differentiated cells.
4. What is the difference between a differentiated and undifferentiated cell?
5. What does cloning add to our definition of an "experimental control plant"?

ALTERNATIVE

1. What are the ethical ramifications of cloning?
 - a. Do you own a clone?
 - b. What is the relationship of the clone to the original organism?
2. Should a human be cloned? If not, can you think of one exception where it could be?
3. What are the possible benefits of cloning human tissues and organs?
4. If we took plants that were cloned and changed their environments, what do you think would happen?
5. Do you think that in college or graduate school you will be cloning the animal kingdom? Why or why not?

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