

# Plant cloning

<b>Focus question</b>	How might we create a sterile growing environment? How important is a sterile environment? How is a plant clone produced?
<b>Vocabulary</b>	Plant tissue, tissue culture, sterile, aseptic technique, explant, nutrient medium

Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or multiple copies of cloned plants. This can be accomplished through the **tissue culture** of small tissue pieces from the plant of interest. These small pieces may come from a single mother plant or they may be the result of a genetic transformation of single plant cells, which are then encouraged to grow and to ultimately develop into a whole plant. Tissue culture techniques are often used for commercial production of plants as well as for plant research.

Tissue culture involves the use of small pieces of plant tissue (**explants**), which are **cultured** in a **nutrient medium** under sterile conditions. Using the appropriate growing conditions for the explant type, plants can be induced to rapidly produce new shoots and new roots. These plantlets can also be divided, usually at the shoot stage, to produce large numbers of new plantlets. The new plants can then be placed in soil and grown in the normal manner.

The most important part of this activity, however, is to maintain a **sterile environment** and use **aseptic technique** to control possible contamination. Even one fungal spore or bacterial cell that comes into contact with the growth media will rapidly reproduce and soon completely overwhelm the small plant.

## Materials

- Sterile distilled water
- 10% bleach solution (Mix 100 mL household bleach + 900 mL water)
- 70% alcohol
- Cutting equipment such as a scalpel blade or razor blade  
(Sterile or dip in 10% bleach then rinse in sterile water)
- Forceps or tweezers (Sterile or dip in 10% bleach then rinse in sterile water)
- Gloves
- Cauliflower florets
- Sterile paper towels or petri dishes for cutting
- Lidded containers in which to wash the plant material
- Detergent-water mixture (Mix 1ml detergent per liter of water)
- Sterilizing solution: 1–2% bleach Solution (Mix 5–10 ml household bleach + 500 ml water)
- 2 or 3 beakers or jars for sterile water
- 2 spray bottles

## Procedure

1. Mix 10% bleach solution, detergent-water mixture and sterilizing solution using amounts given.
2. Add 10% bleach to a spray bottle and add some to a beaker or jar for rinsing.
3. Add 70% alcohol to the other spray bottle.

## **Plant preparation**

You will be sterilizing the plant material to remove any bacteria or fungal spores that are present. The aim is to kill all microorganisms, but at the same time not cause any adverse damage to the plant material.

1. Cut cauliflower into small sections of florets about 1 cm across on a sterile surface within the sterile transfer chamber. If using plant leaves, cut into disks with a sterilized cork borer.
2. Wash the prepared plant material in the detergent-water mixture for about 20 minutes. This will help remove fungi etc., and the detergent will help wet the material and remove air bubbles that may be trapped between tiny hairs on a plant.
3. Transfer the washed plant material to the sterilizing solution. Shake the mixture for 1 minute and then leave to soak for 10–20 minutes. Carefully pour off the bleach solution using the lid to keep the plant tissue from coming out and then carefully cap the container.

*Note:* At this point, the tissue is considered sterile. All subsequent rinses should be done with sterile water and all manipulations of the tissue performed with sterile instruments and supplies. Open one container at a time and never leave the lid off of any container longer than necessary.

## **Transfer of plant material to tissue culture medium**

1. Use sterile gloves and equipment for all of these steps.
2. Spray the outside surfaces of the containers, the capped tubes, and the aluminum-wrapped supplies with 70% alcohol before moving them into the chamber.
3. Place the plant material still in the bleach sterilizing container, the containers of sterile water, the sterilized forceps and blades, some sterile paper towels to use as a cutting surface, and enough tubes containing sterile medium into the sterile area.
4. Spray the gloves with a 70% alcohol solution and rub hands together to spread the alcohol just prior to placing hands into the chamber. Once gloves are on and sprayed, they must not touch anything that is outside of the sterile chamber.
5. Carefully open the container with the plant material and pour in enough sterile water to half-fill the container. Replace the lid and gently shake the container to wash tissue pieces (explants) thoroughly for 2–3 minutes to remove the bleach. Then let sit for 15 minutes. Pour off the water.
6. Remove the sterilized and washed plant material from the container, then place on the paper towel or sterile petri dish. Cut the cauliflower into smaller pieces about 2 to 3 mm across. Be sure to avoid any tissue that has been damaged by the sterilizing solution, which is apparent by its pale color. If using a disk of plant leaf, no additional cutting is necessary.
7. Take a prepared section of plant material in sterile forceps and place into the medium in the culture tube. Cauliflower pieces should be partly submerged in the medium, flower bud facing up. If using another plant leaf, be sure the disk is in contact with the agar.
8. Replace the lid on the culture tube.

Growing the plants

- 1. Place plant sections in a well-lit area of the classroom although not in direct sunlight. The shoots will grow more quickly if the explants are placed under fluorescent or grow-lights to provide at least 12 hours of light per day. The aquarium can be used as a growth chamber with the lighting about 8–10" overhead. This will also help maintain a more regular and warm temperature. Ensure that the temperature does not go over 82.4° F. New shoots should develop within 2 weeks and should be well advanced in 3 to 4 weeks. Check the tubes daily and discard any that show signs of infection (before discarding, add bleach into the tube). Roots can appear within 6 weeks on cauliflowers.

Rubric for self-assessment

Skill	Yes	No	Unsure
It was easy to use sterile technique to sterilize and transfer explants to culture tubes.			
The explants grew into healthy plants.			