

# E X E R C I S E

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## Corn Embryo Culture

In this experiment, you will surface-sterilize a part of a corn cob. You will then cut off the kernels and extract the embryo. The embryo will be placed on a plate of solidified MS medium with 2mg/L 2,4-D. The 2,4-D is an auxin that stimulates callus formation.

You will grow these embryos in light at room temperature and observe what happens to the embryos. If the embryos look as if they are growing a root and shoot, they probably are! In this case, it means that the embryo was too old to dedifferentiate into generic cells.

The embryo is the little white thing that squirts out of each kernel of corn when you eat it. The round side is the scutellum, which is the cotyledon of the corn seed. The scutellum absorbs nutrition from the endosperm for the embryo. But since the endosperm is removed, the embryo absorbs all its own nutrition from the medium.

### PROCEDURE

1. Spray beakers with 70% Ethanol to surface-sterile them; do not remove foil yet. Place inside a laminar flow hood, and then remove foil. Fill 1 beaker with 70% Ethanol, 1 beaker with 20% bleach, and the other 2 beakers with sterile ddH<sub>2</sub>O.
2. Place your corn cob in the Ethanol and sterilize for 5 minutes. Remove from Ethanol and place in bleach for 5 minutes. Rinse cob in water 2 x 5 minutes.
3. Making sure your hands are sterile, cut kernels off cob with the scalpel blade. Cut close enough to the cob so that you won't cut the embryo in half, but so that you have cut the bottom of the kernel off. Gently place forceps on the side of an excised kernel to push out embryo.
4. Gently pick up embryo with 1 tine of forceps and place it flat side down on the plate of medium. The embryo has a flat side and a round side. Do not squash the embryo between the tines of the forceps. Do not push the embryo into the medium, as this will suffocate it.
5. When you have excised 8-10 embryos, cover the plate and wrap with parafilm. Label your plate with your name, date, and lab number. Place the plate in light. Observe over the next few weeks, and record your observations.
6. Now let your lab partner excise 8-10 embryos.

## ANTICIPATED RESULTS

The embryos should grow in size and become crumbly, granular, whitish-yellow masses of cells called a callus. If calli do form, transfer them to fresh medium after two weeks and continue to observe.

The younger the cob, the more likely calli will form. If you need to purchase a cob from a grocery store, for example, use the tip, since embryos are progressively younger as you move along the cob toward the tip.

## MATERIALS

4 sterile, autoclaved beakers per pair

70% Ethanol

20% bleach

Sterile ddH<sub>2</sub>O

Sterile forceps

Scalpel blades

Corn cob

2 plates of MS medium with 2mg/L 2,4-D (medium is MS medium from Sigma (M-9274) powder with micro- and macro-nutrients, vitamins, sucrose, and agar)

Parafilm