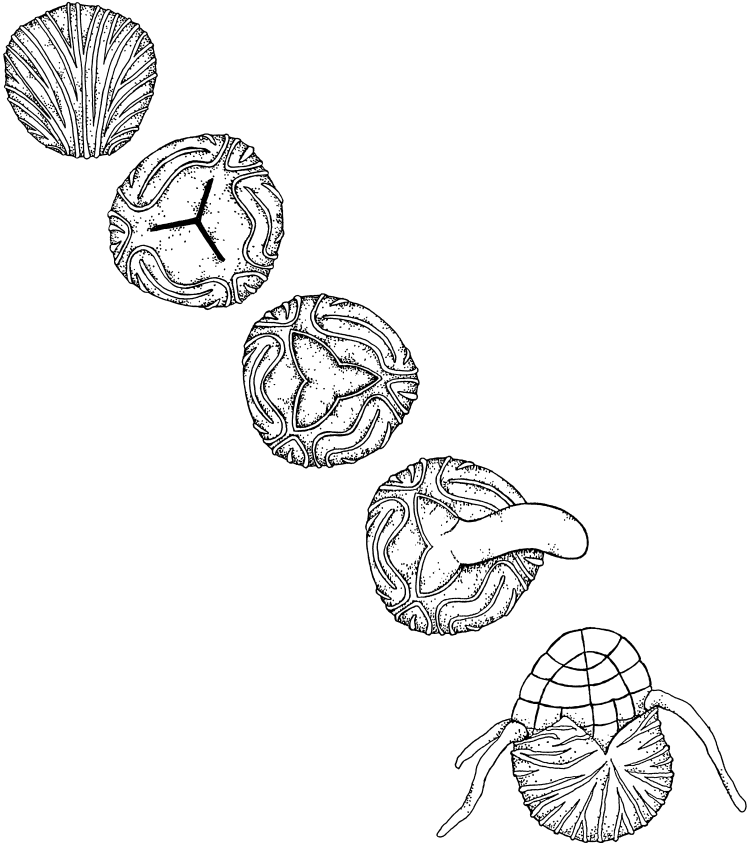


# C-Fern<sup>®</sup> Spores

Instructions



**CAROLINA**

*World-Class Support for Science & Math*

To grow *C-Fern*<sup>®</sup> successfully, you will need

- an appropriate growing environment
- a light source
- a medium
- spores

## Growth and Development

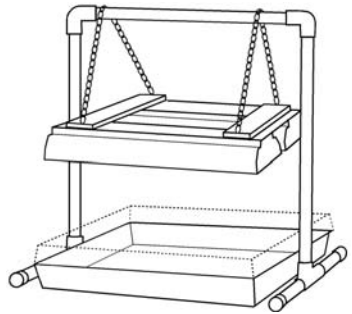
Plant-growing systems can be easily adapted to provide adequate environmental conditions for *C-Fern*. To attain the optimum growth and timing of development as indicated in *C-Fern* kits, cultures require adequate lighting and temperature control. If your temperature and lighting conditions differ substantially from that indicated, a test run should be carried out to determine when, under your conditions, specific developmental stages will be present for observation and manipulation. Two options, **Culture Domes** and **Growth Pods**, available through Carolina Biological Supply, are described. Note: When using either option, do not tightly seal the petri dishes (e.g., using Parafilm<sup>®</sup>).

### **Culture Domes** (RN-15-6792)

Culture Domes, consisting of greenhouse trays covered with transparent humidity domes, regulate temperature and humidity, reduce contamination, and permit easy handling of a larger number of dishes. An illustration of a Culture Dome with an appropriate lighting stand is shown below.

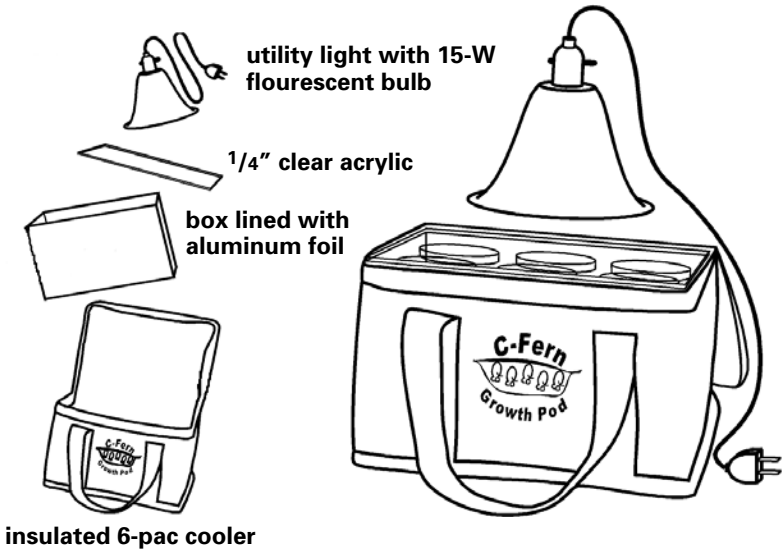
Maintain your *C-Fern* cultures under continuous illumination using 40-W cool-white fluorescent light tubes at a distance of 45 cm or less from the cultures. Vary the distance between the lights and the Culture Dome to obtain a temperature near the 28°C optimum. Larger lighting stands will accommodate additional Culture Domes.

**Remember:** The correct temperature inside the Culture Dome is more important than a specific level of light.



## Growth Pods (RN-15-6715)

The Growth Pod makes culturing *C-Fern* even easier, more reliable, cheaper, and faster by reducing needed space and allowing better control of culture temperature. When the optimum culture temperature of 28°C is difficult to attain using a Culture Dome, the Growth Pod can dramatically decrease culture time and increase yield.



A simple lighting fixture with a 6" dome and switch (e.g., a small clamp/utility light) and a 15-W screw-in fluorescent bulb are recommended. (These bulbs are long lasting, highly efficient, and feel only warm when left on continuously.) The light may be rested on small blocks (e.g., Legos®) on top of the acrylic lid, or suspended over the top of the lid. Adjust the height to achieve a temperature of 26–30°C within the pod. Small digital thermometers can keep track of the temperature.

The Growth Pod can hold enough petri dishes for a class of 30 or more (six stacks of five 60 × 15-mm petri dishes). As a result of stacking the petri dishes, cultures on the bottom may be developmentally behind those on the top. To minimize this, reverse the order of the stacks after 6–7 days.

For transport, zip the top of the pod closed to reduce temperature and humidity fluctuations.

## Temperature

- The optimum temperature for spore germination and gametophytic development is 28°C (82°F), but results are good at any temperature within the range of 26–30°C (79–86°F).
- Lower temperatures will substantially alter development.
- Monitor and record temperatures inside the Growth Pod/Culture Dome daily.
- Adjust the distance between the light and the Growth Pod/Culture Dome to control temperature. Once a suitable temperature is achieved, the height of the light should remain constant.

### *Troubleshooting—Condensation*

Condensation on petri dish lids may occur if there is excessive temperature variation. Moderate amounts of condensation are not a problem, but excessive condensation may result in an uncontrolled release of spermatozoids. If condensation becomes a problem, grow cultures upside down once the sowing water has been absorbed in the medium.

## Preparing the Medium

**Bottled Basic *C-Fern*<sup>®</sup> Medium** (RN-15-6780, 160 mL; RN-15-6781, 400 mL)

### 1. Melt the medium.

(To speed up this process, shake the bottle to break up the medium.)

- Loosen the cap on the container.
- Place the container in a hot water bath such that the water level is just above the level of the medium.
- Do not submerge the container!

### 2. Pour the medium into the petri dishes.

- Medium should be poured in a clean area free from drafts and traffic.
- Wipe down the area with 70% ethanol or isopropanol.
- Open a sleeve of 60-mm petri dishes.
- Check that the medium is completely melted.
- Gently swirl the medium to ensure it is mixed.
- Tilt the lid of a petri dish just enough to permit pouring.
- Fill the dish about  $\frac{3}{4}$  full (about 15 mL per dish). Note: Do not underfill the dishes.

- 160 mL of medium yields 10 petri dishes; 400 mL yields 25 dishes.
- Replace the petri dish lid and allow the dish to cool.

**Powdered Basic C-Fern® Medium (RN-15-6782)**

To prepare 1 L of medium:

- Open the packet and add powder to about 800 mL of distilled water in a volumetric flask.
- Rinse out the remaining powder with distilled water.
- Stir until the powder is completely dissolved.
- Bring to a final volume of 1 L.
- Add 10 g Difco Bacto® agar.
- Adjust the pH to 6.0 with 1.0 N NaOH.
- Autoclave at 120°C/20 psi for 15 minutes.
- Dispense into sterile petri dishes. Be sure to fill each dish about 3/4 full.

If an autoclave is not available, a microwave can be used to prepare the medium. Use caution when heating and handling. Wear safety goggles, use gloves, and do not leave the microwave unit unattended.

**Steps for using a microwave to prepare 1 L of medium.<sup>1</sup>**

<b>Step</b>	<b>Microwave Time (1000-W output unit)</b>	<b>State of Solution<sup>2</sup></b>	<b>Post-Mircrowave Procedure<sup>3</sup></b>
<b>1</b>	<b>5 minutes</b>	<b>hot</b>	<b>remove, swirl</b>
<b>2</b>	<b>1 minute</b>	<b>boiling</b>	<b>remove, swirl</b>
<b>3</b>	<b>15 seconds</b>	<b>boiling</b>	<b>remove, swirl</b>
<b>4</b>	<b>15 seconds</b>	<b>boiling</b>	<b>remove, swirl</b>
<b>5</b>	<b>15 seconds</b>	<b>boiling</b>	<b>swirl to mix and pour petri dishes</b>

<sup>1</sup> Use a vessel at least twice the volume of the medium.

<sup>2</sup> The solution will boil vigorously in steps 2–5. The medium must be visually monitored constantly so that if it starts to boil over, the power can be reduced or turned off briefly.

<sup>3</sup> It is important to thoroughly mix the medium by swirling the flask after each step and while pouring the plates.

# Preparing Spores

Tap the vial on a hard surface so that the spores are at the bottom of the vial before opening it.

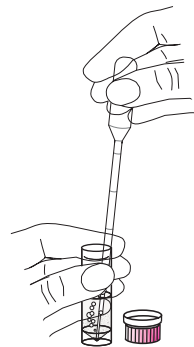
## Presterilized Spores

- Loosen the cap.
- Transfer the appropriate amount of sterile distilled water to the spore vial with a sterile pipet (the standard presterilized spore unit will sow about 35 dishes at a density of 300+ spores per plate when 4 mL of sterile distilled water is added).
- Do not return any water to the sterile water bottle.
- Wet the spores completely by firmly tightening the cap and inverting the vial 2–3 times.
- With the cap on, check the bottom of the vial to ensure that all spores have been suspended.
- Return the vial to the holder and loosen the cap.

## Unsterilized Spores

Sterilize spores according to the following procedure:

- Weigh the spores on glassine weigh paper and transfer them to a sterile, conical, 5-mL tube. (10 mg will inoculate 35 dishes.)
- Presoak the spores by covering them with 1–2 mL of sterile distilled water for 15 minutes to 24 hours.
- Remove the presoak water:
  - a. Insert a sterile pipet into a conical tube.
  - b. Suspend the spores by bubbling a small amount of air into the water.
  - c. Gently, but securely, seat the pipet tip at the base of the tube.
  - d. Squeeze the bulb to force air out of the pipet.
  - e. Release the bulb to draw water into the pipet while spores collect around the tip.
- Surface sterilize the spores:
  - a. Suspend the spores in 1–2 mL of 0.875% sodium hypochlorite.
  - b. Rinse down the lip and sides of the spore vial with sodium hypochlorite solution.



- c. To evenly suspend the spores, bubble air through a clean, sterile pipet.
- d. Sterilize the spores for 3 minutes.
- e. Remove the sodium hypochlorite solution with a clean, sterile pipet.
- Rinse the spores:
  - a. Add 1 full pipet (2 mL) of sterile distilled water.
  - b. Remove the water with a clean, sterile pipet.
  - c. Repeat 1 or 2 more times.

## Sowing and Spreading Spores

### Sowing Spores

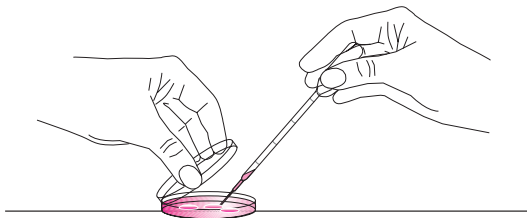
For consistent coverage of the petri dishes, the spores must be suspended before each sowing.

To suspend the spores,

- gently draw the liquid, along with the spores, in and out of a pipet.
- withdraw a small amount of the spore suspension into the pipet and immediately dispense the appropriate number of drops onto the agar (300+ spores = 3 drops of spore suspension per dish).

When sowing,

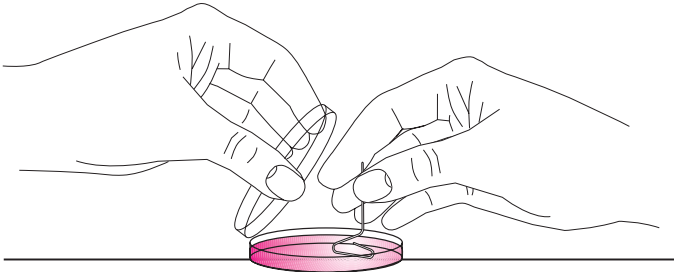
- tilt the lid of the petri dish upward only enough to permit access of the pipet.
- hold the pipet at a constant angle of 45°.
- do not touch the agar surface with the tip of the pipet.
- resuspend the spores between each sowing by gently squeezing and releasing the pipet bulb.



## Spreading Spores

A metal wire spreader (RN-70-3412) or a paper clip bent into the shape of a spreader should be sterilized in a flame and allowed to cool, or simply wiped with 70% alcohol and allowed to dry.

Move the spreader gently back and forth across the surface of the agar while rotating the petri dish slowly with the other hand. Evenly spread spores will result in cultures that grow better and are easier to observe.



Visit the *C-Fern* Web page at [cfern.bio.utk.edu](http://cfern.bio.utk.edu) for additional information on using *C-Fern* materials in teaching and research.

Check Carolina's printed or online catalogs for the full line of *C-Fern* spore stocks and teaching materials.

# Carolina Biological Supply Company

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