



## **Fern Micropropagation**

### **Hints on Successful Tissue Culture Propagation of Fern**

Ferns are generally very easy to propagate through plant tissue culture. Most ferns that are commercially available through garden centers have been produced through plant tissue culture. Many ferns can be started by taking the runner from actively growing plants and culturing them on a medium containing a mixture of essential nutrients, growth regulators and a gelling or solidifying agent.

#### *Materials Required*

1. 10 sterile petri dishes, either glass or plastic (100 mm in diameter) to be used as cutting surfaces.
2. 3 500-ml beakers and one 250-ml beaker
3. 3 pairs of forceps and 2 scalpels
4. 9 plastic racks
5. Waterproof marking pen and labels
6. Glass Bead Sterilizer (Product Number S636) or Alcohol Burner (Product Number B876)
7. 1000 ml of 10% Clorox solution supplemented with a few drops of Tween-20 (Product Number P720)
8. 1000 ml of sterile distilled water
9. Murashige Fern Multiplication Medium (Product Number M508)
10. 12-15 fern runners (10 cm in length)
11. Isopropyl Alcohol

#### *Procedures*

##### Surface Sterilization:

1. Wipe down all surfaces of the transfer hood or glove box with 70% isopropyl alcohol. Allow the hood to run for 15 min before beginning transfer operations. Place all the materials listed in the previous section under the hood. Place scalpels and forceps in a 250-ml beaker containing about 100 ml of 95% ethanol.
2. Select 12-15 Fern runners, 10 cm in length. Rinse the runners under running water then cut into 2.5 cm section and transfer them to the 500-ml beakers. Place the beaker under the hood and pour the Clorox solution over the runners, making certain all surfaces are properly covered. Leave the runners in the sterilization



solution for 10 to 15 minutes and then pour off the solution. Rinse the runners three times in sterile distilled water with each rinse lasting approximately 1 min.

**Media Preparation:**

Stage 1—Use Murashige Fern Multiplication Medium at 1/2x strength (2.33 g/L).

Stage II—Use Murashige Fern Multiplication Medium at full strength (4.66 g/L).

Stage III—Use Murashige and Skoog Basal Medium at 1/2x strength (2.2 g/L) supplemented with 1.0 mg/L IBA.

Product Description	Product Number	Stage I	Stage II	Stage III
Murashige Fern Multiplication Medium	M 508	2.33 g/L	4.66 g/L	
Murashige & Skoog Basal Medium	M 519			2.2 g/L
Sucrose	S391	30.0 g/L	30.0 g/L	20.0 g/L
Agar	A111	7.0 g/l	7.0 g/L	7.0 g/L
Indole-3-Butyric Acid	I460			1.0 mL/L

Powdered media are extremely hygroscopic and must be protected from atmospheric moisture. If possible the entire contents of each package should be used immediately after opening. Preparing the medium in a concentrated form is not recommended as some salt added to the medium may affect shelf life and storage conditions. The basic steps for preparing the culture medium are listed below:

1. Measure out approximately 90% of the final required volume of tissue culture grade water, e.g. 900 ml for a final volume of 1000 ml. Select a container twice the size of the final volume.
2. While stirring the water add the powdered medium and stir until completely dissolved.\*
3. Rinse the original container with a small volume of tissue culture grade water to remove traces of the powder. Add to the solution in Step 2.
4. Add desired heat stable supplements (e.g. sucrose, gelling agent, vitamins, auxins, cytokinins, etc.)
5. Add additional tissue culture grade water to bring the medium to the final volume.
6. While stirring, adjust medium to desired pH using NaOH, HCl, or KOH.

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7. If a gelling agent is used, heat until the solution is clear.
8. Dispense the medium into the culture vessels before (or after) autoclaving according to your application. Add heat labile constituents after autoclaving.
9. Sterilize the medium in a validated autoclave at 1 kg/cm<sup>2</sup> (15 psi), 121°C, for the time period described under Sterilization of Media.
10. Allow medium to cool prior to use.  
\*Heating may be required to bring powders into solution.

Procedures:

1. Take the sterilized fern runners and place them one at a time on the sterile cutting surface.
2. Remove ¼" from the cut end of the runners.
3. Place the runners on the culture medium. You can place 2-3 runners per tub.
4. Seal the tubes and place under 100-300 f.c from fluorescent light with a 16 hours light/8 hour's darkness.
5. Explants in Stage I may turn black or brown if they were exposed to too much sterilization. If all cultures turn brown, repeat the sterilization procedures but reduce the time in the sterilization solution by 1/3. With 15-30 days you should begin to see new growth coming from the runners that were not damaged by the sterilization..
6. After 45 days, transfer the ferns to fresh media. To increase the number of plants, transfer to Stage II media. To root the ferns and prepare them for transferring to soil, use Stage III media.
7. Cultures in Stage II should be transferred to fresh media every 30 days.

*Scheduling*

<b>Event</b>	<b>Timing</b>
Isolation of fresh explants	Day 0
First appearance of organogenesis	ca. Day 14
Noticeable shoot formation	ca. Day 30
First subculture	ca. Day 45

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