

Tissue Culture  
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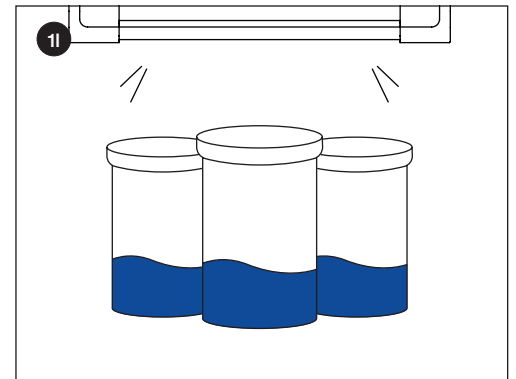
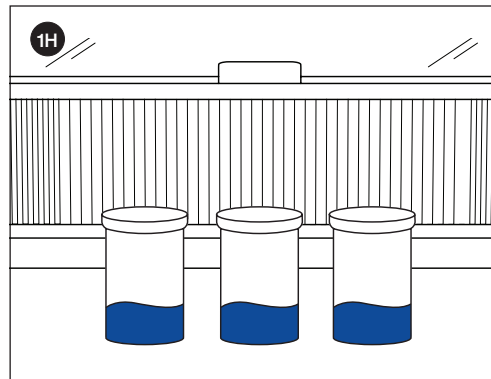
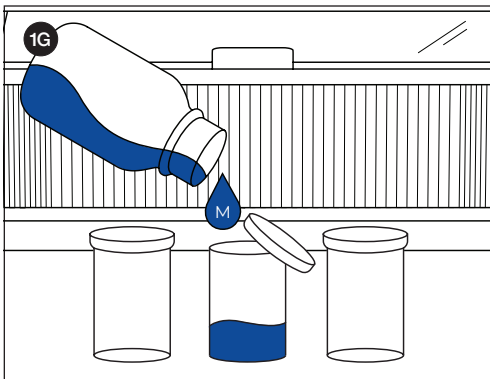
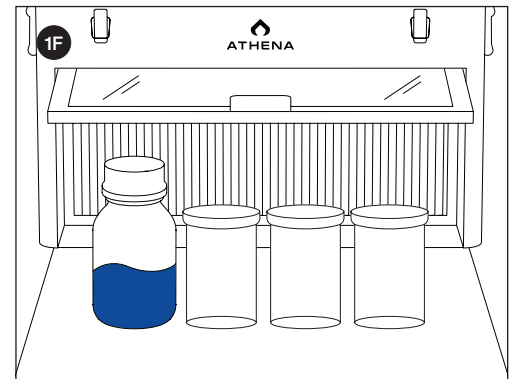
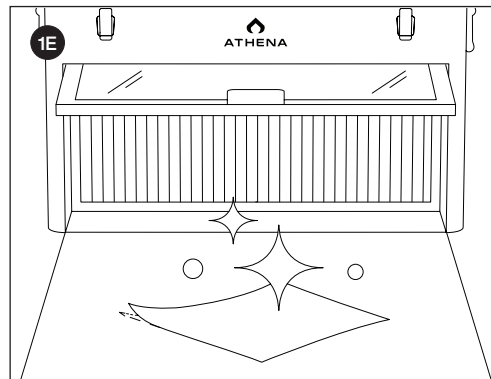
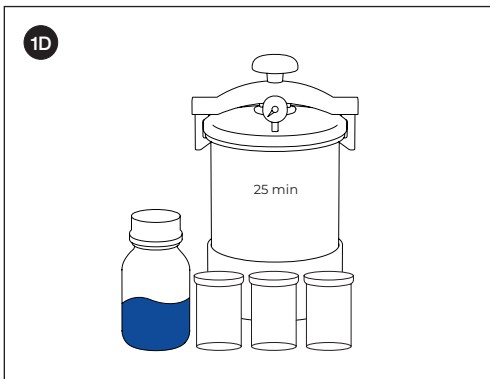
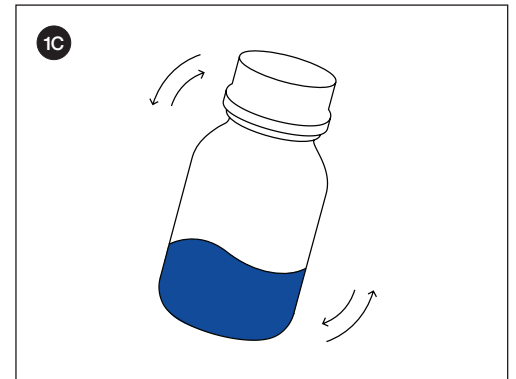
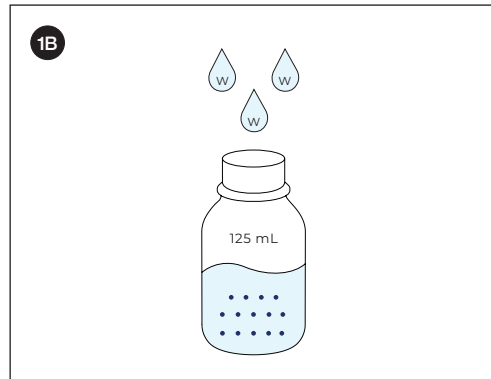
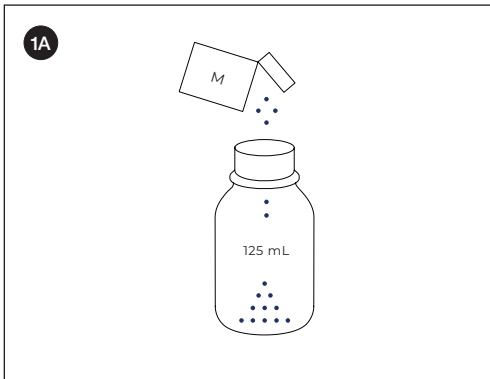


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- All sterile work is to be done under the **workzone** unless stated otherwise.
- Always wear gloves and a face covering when working in the **workzone**.
- Any container with liquid placed in the **Autoclave** must have a loosely opened lid.
- Spray gloves with alcohol between processes in the **workzone**.

**STEP 1: MEDIA PREP**

- 1A.** Pour one pack of 125 mL Shoots or Roots media powder (M) into the 250 mL media vessel.
- 1B.** Pour filtered water (W) into the media vessel up to 125 mL and close the lid.
- 1C.** Agitate the media vessel until the solution is fully dissolved.
- 1D.** Place the desired amount of culture vessels and the media vessel into the Autoclave for a full cycle.
- 1E.** Clean and sterilize the Flow Hood workzone with alcohol wipes (inside and front surfaces).
- 1F.** Place the items from the Autoclave directly under the Flow Hood and allow to cool until roughly 113° - 130°F or as soon as possible to handle.
- 1G.** Before solution turns to gel, fill each culture vessel ¼ full with media solution (M) and cap.
- 1H.** Let culture vessels settle until the media turns into a gel.
- 1I.** Set culture vessels back into the toolbox of the Flow Hood or in a cool dark place.



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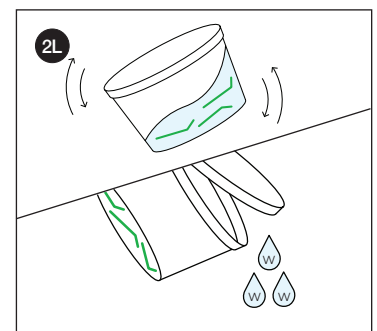
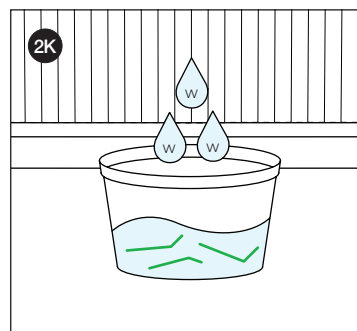
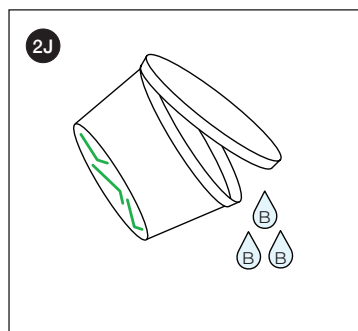
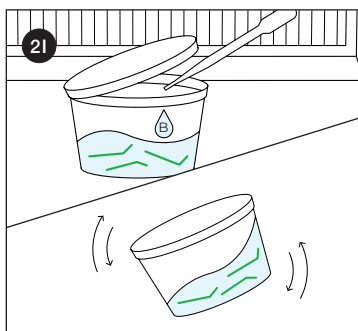
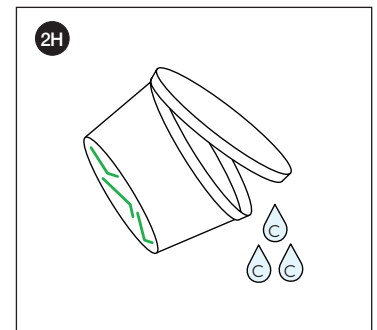
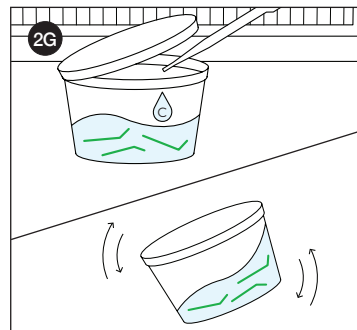
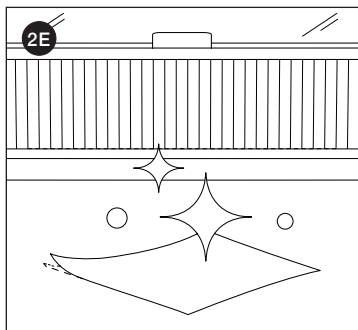
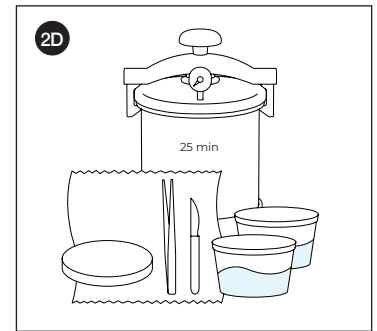
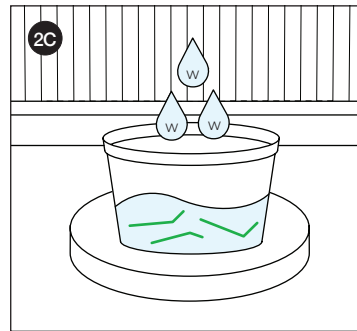
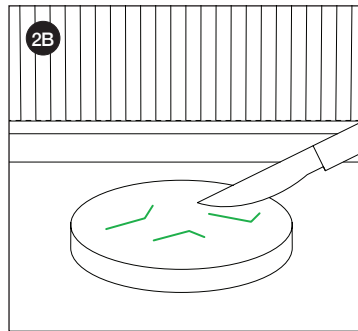
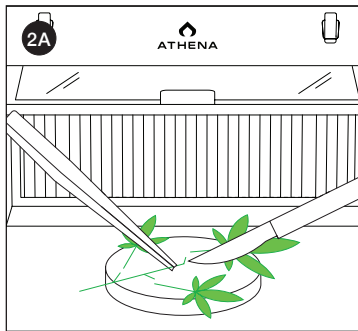
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- Always wear gloves and a face covering when working in the **workzone**.
- Any container with liquid placed in the **Autoclave** must have a loosely opened lid.
- Spray gloves with alcohol between processes in the **workzone**.

**STEP 2: PLANT PREP**

- 2A.** Place an apical cut taken from clean, healthy moms on a sterilized **work surface** in the **workzone** and remove the leaf material with a sterilized **scalpel**. **Note:** These can be uppers, lowers or middles. Healthier material will yield best results.
- 2B.** Dissect the cutting at the middle of each internode leaving enough stem under each node to go into the media.
- 2C.** Place all the nodes into a sterilized **utility vessel** and fill it with 8 oz of filtered water (W).
- 2D.** Wrap the **forceps, scalpel, paper towel, and work surface** in aluminum and gather (2) **utility vessels** each filled with 8 oz of filtered water to place into the **Autoclave** for a full cycle.
- 2E.** Clean and sterilize the **workzone** with alcohol wipes (inside and front surfaces).
- 2F.** Place the items from the **Autoclave** into the **workzone** along with the sterilized **utility vessel** filled with nodes.

- 2G.** Add 0.5 mL of **Cleanse (C)** and agitate the mixture for 15 secs.
- 2H.** Slightly open the lid to pour the **Cleanse (C)** solution into a waste container without dropping nodes. **Note:** The waste container is held outside of the **workzone**.
- 2I.** Use the **utility vessel** from the **Autoclave** to refill the **utility vessel** containing the nodes with 8 oz of water, add 20 mL of **Bleach (B)**, agitate the mixture, and leave for 10 min in the **workzone**.
- 2J.** Slightly open the lid to pour the **Bleach (B)** solution into a waste container without dropping nodes. **Note:** The waste container is held outside of the **workzone**.
- 2K.** Use the **utility vessel** from the **Autoclave** to refill the **utility vessel** containing the nodes with 8 oz of water (W).
- 2L.** Agitate the **utility vessel** and pour out the water into a waste container as a final rinse.



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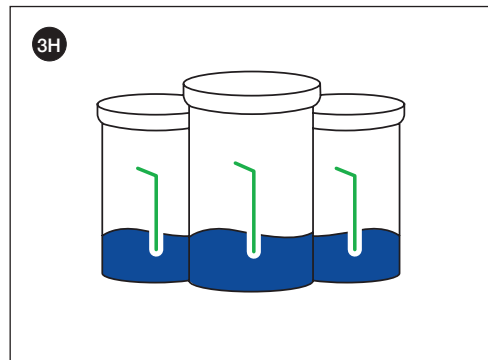
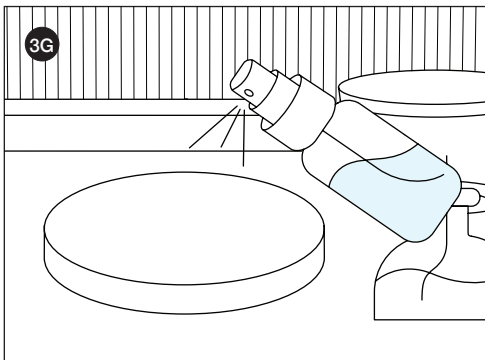
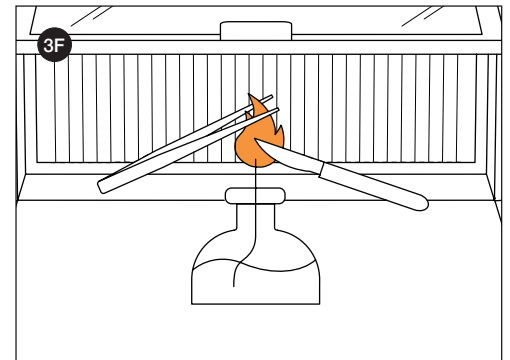
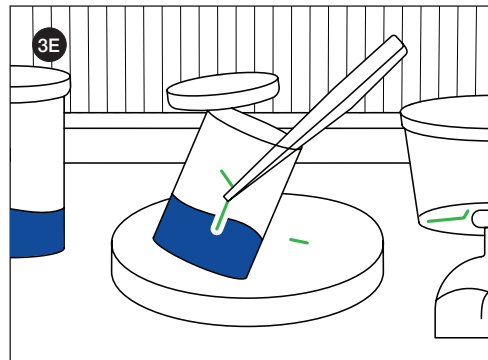
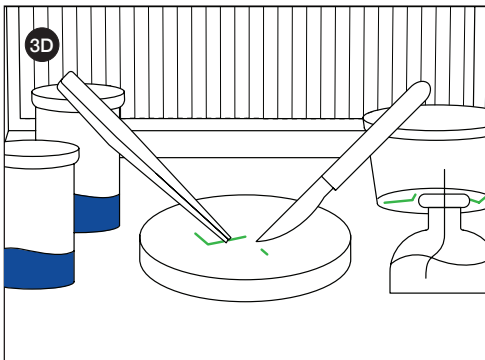
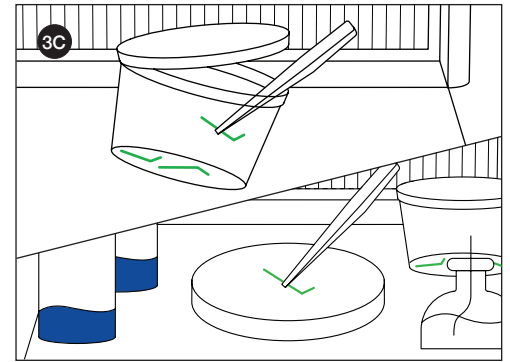
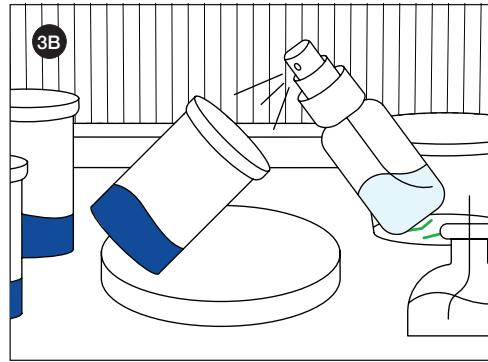
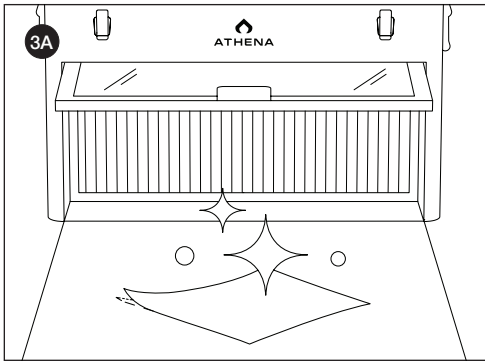


**STEP 3: LAB PREP**

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- Always wear gloves and a face covering when working in the **workzone**.
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- Spray gloves with alcohol between processes in the **workzone**.

- 3A.** Clean and sterilize the **workzone** with alcohol wipes (inside and front surfaces).
- 3B.** Spray alcohol to sterilize the culture vessels with **Shoots media** and the **alcohol burner** before placing them into the **workzone**.
- 3C.** Slightly open the **utility vessel** with cuttings, remove one node with sterilized **forceps**, and place it on the sterile **work surface**.
- 3D.** While holding the node steady with **forceps**, dissect it at the lower end, leaving enough stem to go into media.
- 3E.** Slightly open the **culture vessel** and place the node's exposed tissue into the media and close the cap right away. This is now an explant. **Note:** Ensure that the tools do not touch media or **culture vessels**.
- 3F.** Sterilize the **forceps** and scalpel blade with the **alcohol burner** after each cutting.
- 3G.** Spray the **work surface** with alcohol after each cutting. **Note:** Repeat steps D-G for each explant you want to create.
- 3H.** When all **culture vessels** are prepared with newly made explants, store them under a clone light at 75-125 PPFD and room temperature of 68 - 78°F



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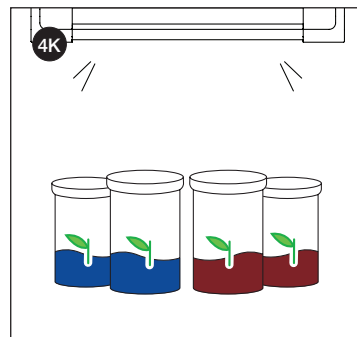
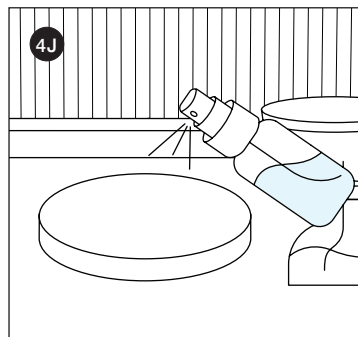
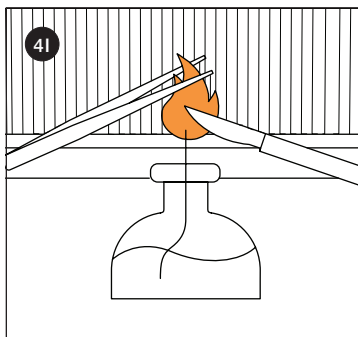
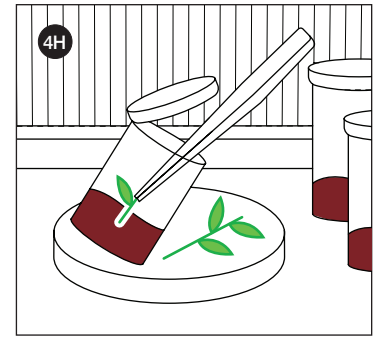
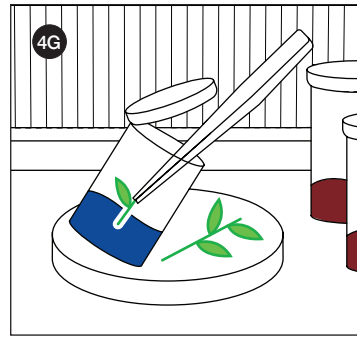
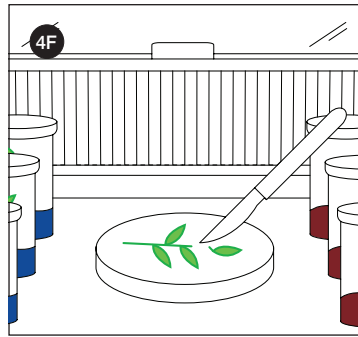
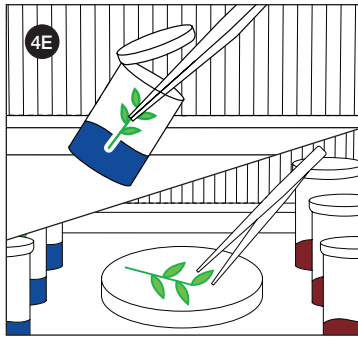
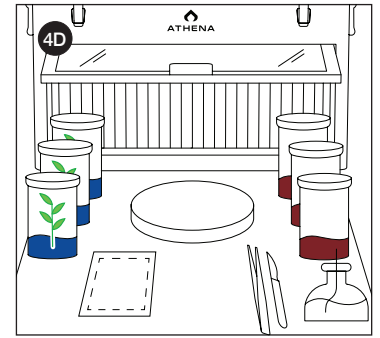
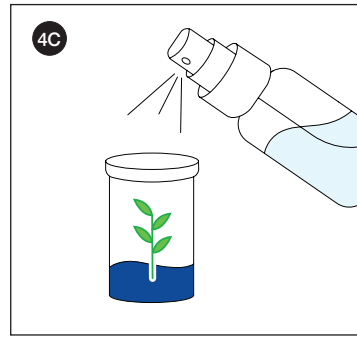
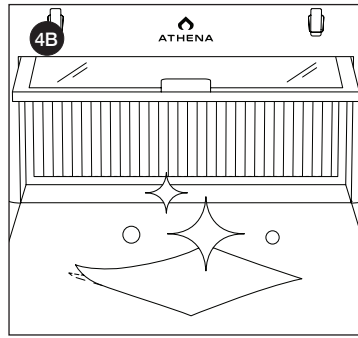
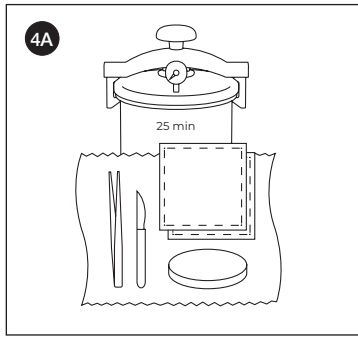
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- Spray gloves with alcohol between processes in the **workzone**.

**STEP 4: TRANSFER**

- 4A.** Wrap the **forceps, scalpel, work surface, and paper towel** in aluminum foil to place in the **Autoclave** for a full cycle.
- 4B.** Clean and sterilize the **Flow Hood workzone** with alcohol wipes (inside and front surfaces).
- 4C.** Use the alcohol sprayer to sterilize all **culture vessels** filled with **Roots/Shoots** formula, the vessels with explants, and the **alcohol burner** before placing them into the **workzone**.
- 4D.** Place the items from the **Autoclave** directly under the **Flow Hood**.
- 4E.** Remove an explant showing prominent new growth from the **Shoots culture vessels** and place it on the **work surface**.
- Note:** Ensure that the tools do not touch the media or **culture vessels**.

- 4F.** Dissect the explant at the middle of each internode leaving enough stem under each node to go into the media.
- 4G.** If the goal is to preserve the genetic material: Place the cutting into a **culture vessel** with **Shoots** media.
- 4H.** If the goal is to create a mother plant: Place the cutting into a **culture vessel** with **Roots** media.
- 4I.** Sterilize the **forceps** and **scalpel** blade with the alcohol burner after each cutting.
- 4J.** Spray the work surface with alcohol after each cutting.  
**Note:** Repeat steps 4E-4J for each explant dissected.
- 4K.** When all **culture vessels** are prepared with newly made explants, store them under a clone light at 75-125 PPFD and 68-78°F room temperature.



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