ATHENA®

CULTURE LINE PROCEDURES

CULTURE

Tissue Culture Procedure Video:

STEP 1: MEDIA PREP

⚠ DISCLAIMER:

- 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**.

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.

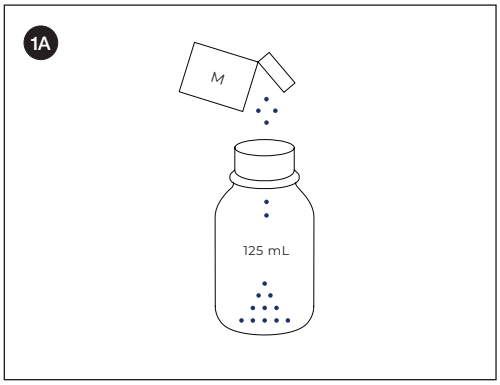
1E. Clean and sterilize the **Flow Hood workzone** with alcohol wipes (inside and front surfaces).

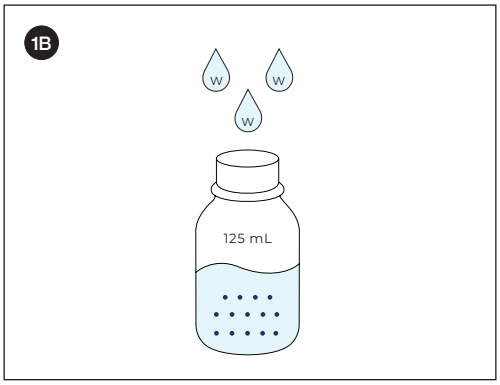
1F. Place the items from the **Autoclave** directly under the **Flow Hood** and allow to cool until roughly 113° - 130°F or as soon as possible to handle.

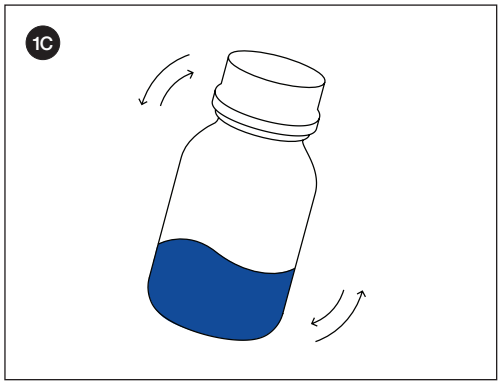
1G. Before solution turns to gel, fill each **culture vessel** ¼ full with **media solution (M)** and cap.

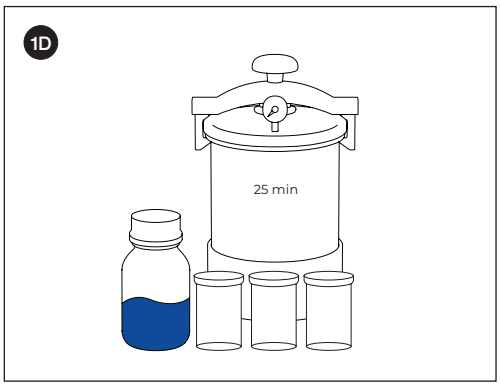
1H. Let **culture vessels** settle until the media turns into a gel.

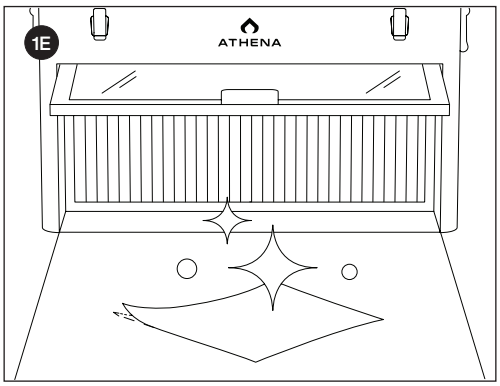
1I. Set **culture vessels** back into the **toolbox** of the **Flow Hood** or in a cool dark place.

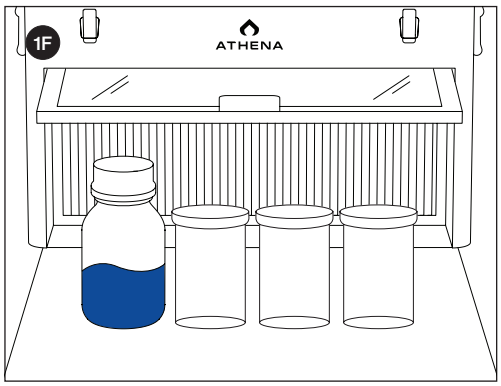


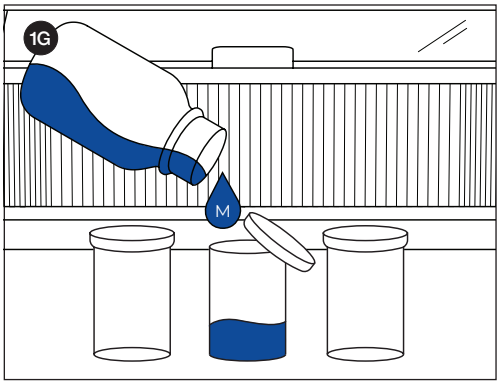


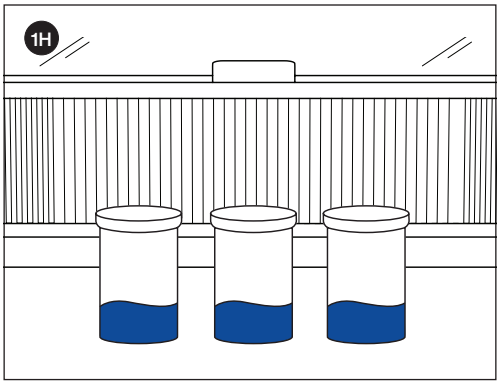


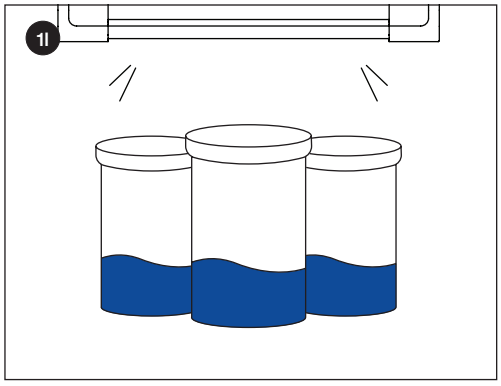












ATHENA®

CULTURE LINE PROCEDURES

CULTURE

Tissue Culture Procedure Video:

STEP 2: PLANT PREP

⚠ DISCLAIMER:

- 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**.

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**.

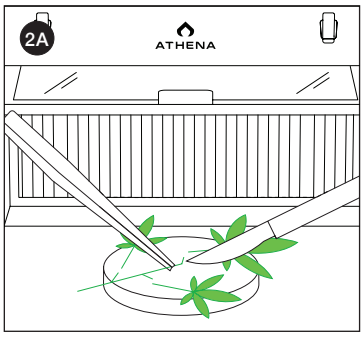
2H. Slightly open the lid to pour the **Cleanse (C)** solution into a waste container without dropping nodes. **Note:** The waste container is held outside of the **workzone**.

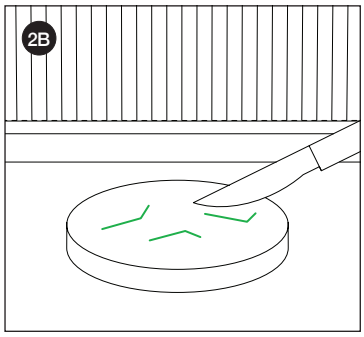
2I. Use the **utility vessel** from the **Autoclave** to refill the **utility vessel** containing the nodes with 8 oz of water, add **20 mL** of **Bleach (B)**, agitate the mixture, and leave for **10 min** in the **workzone**.

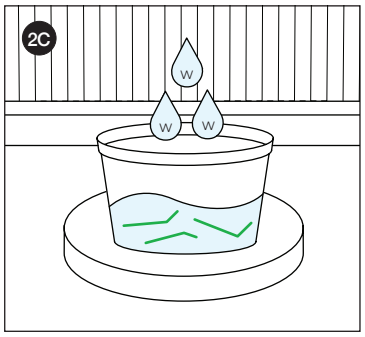
2J. Slightly open the lid to pour the **Bleach (B)** solution into a waste container without dropping nodes. **Note:** The waste container is held outside of the **workzone**.

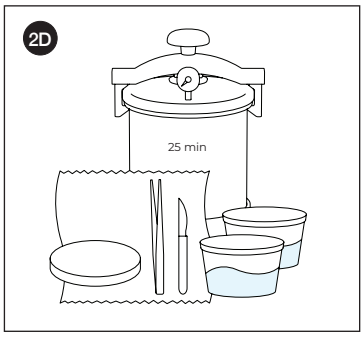
2K. Use the **utility vessel** from the **Autoclave** to refill the **utility vessel** containing the nodes with 8 oz of water (W).

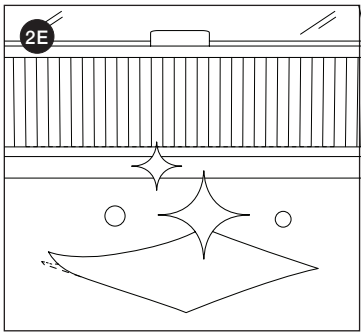
2L. Agitate the **utility vessel** and pour out the water into a waste container as a final rinse.

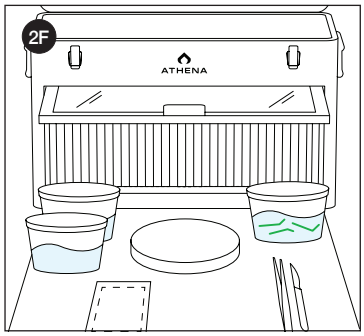


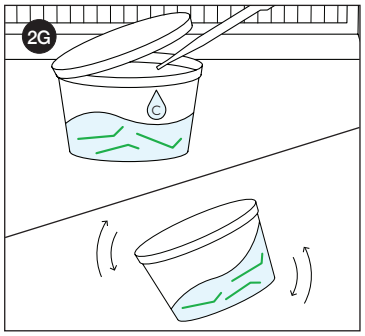


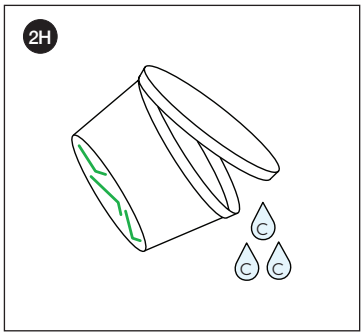


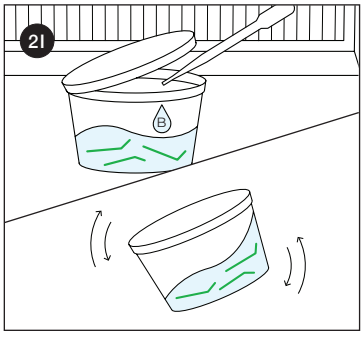


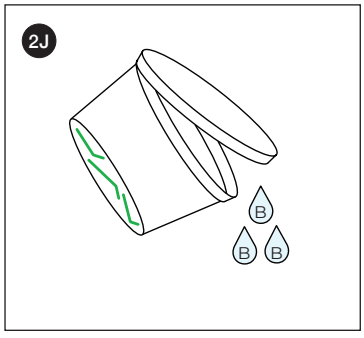


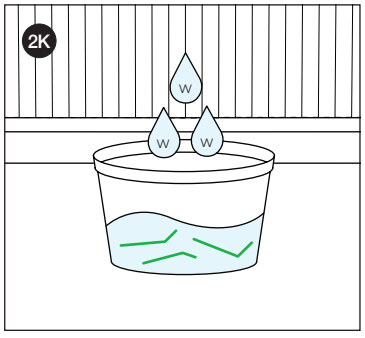


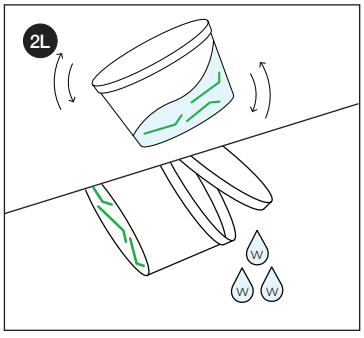












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Tissue Culture Procedure Video:

STEP 3: LAB PREP

⚠ DISCLAIMER:
- 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**.

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.

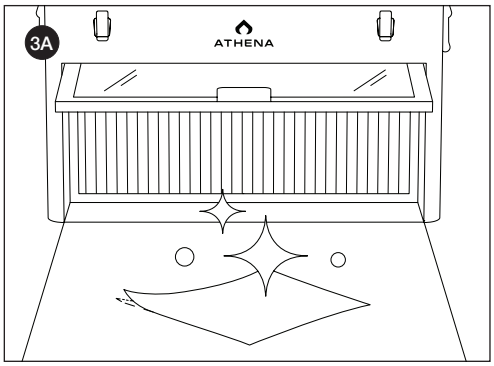
3E. Slightly open the **culture vessel** and place the node's exposed tissue into the media and close the cap right away. This is now an explant. **Note:** Ensure that the tools do not touch media or **culture vessels**.

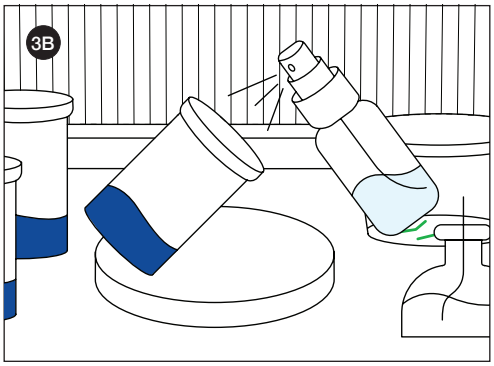
3F. Sterilize the **forceps** and scalpel blade with the **alcohol burner** after each cutting.

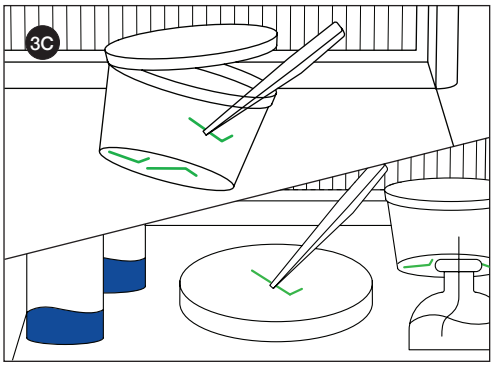
3G. Spray the **work surface** with alcohol after each cutting.

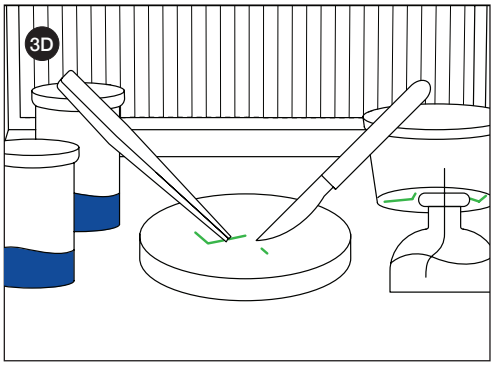
Note: Repeat steps D-G for each explant you want to create.

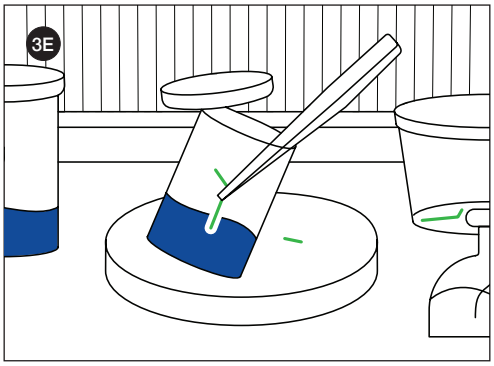
3H. When all **culture vessels** are prepared with newly made explants, store them under a clone light at 75-125 PPFD and room temperature of 68 - 78°F

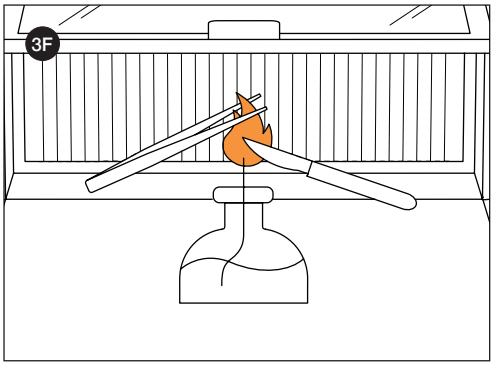


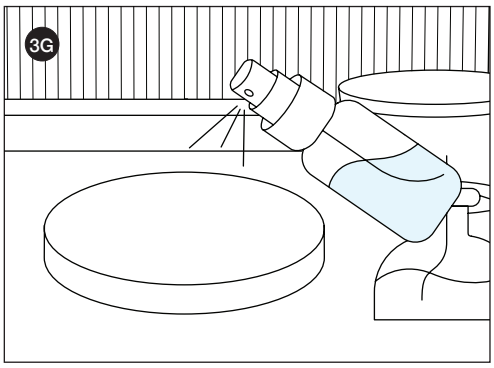


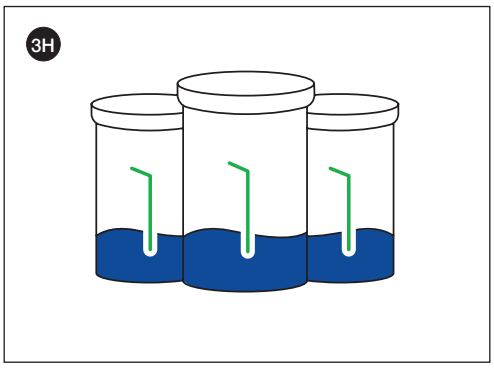












ATHENA®

CULTURE LINE PROCEDURES

CULTURE

Tissue Culture Procedure Video:

STEP 4: TRANSFER

⚠ DISCLAIMER:

- 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**.

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.