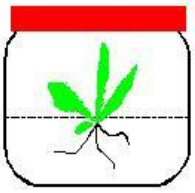


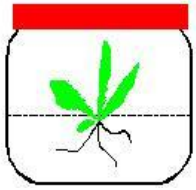
Kitchen Culture Kits Inc.

presents

Plant Tissue Culture

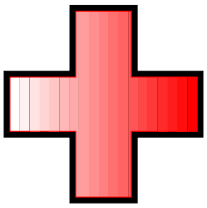
for the Hobbyist, Teacher, Nurseryman and All Plant Lovers

(Includes details on our "Basic" and "Workshop/Classroom Ready" Kits)

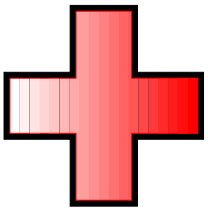


- Plant tissue culture involves the growth of plant parts in a sterile environment for the purpose of mass production.
- Through the use of plant growth regulators, small plant parts can be induced to produce hundreds of small "plantlets" which can be further developed and grown in greenhouses or as house plants.
- Using a microwave oven or a pressure cooker, supplies found in your kitchen, plus supplies provided at this workshop, you can mass propagate hundreds of your favorite plants in your kitchen or classroom.
- In this workshop you will make your own media, assemble a "clean box", disinfect and culture plant leaves, axillary buds and seeds, and discuss trouble shooting and resources.

Tentative Workshop Schedule	
9:00	▸ Introductions ▸ Safety and food.....**PAGE 2** ▸ Location of aprons, restrooms, vending machines, building exits ▸ **Please turn off cell phones and remove hats**
9:30	Plant Tissue Culture for Hobbyists, Teachers and "All Plant Lovers" PPT1
10:30	"Break"
10:45	Media preparation using a microwave: Instructor will assist students in preparation of media using a microwave and "kitchen" vs. scientific methods.....**PAGE 9** Assemble PVC boxes, prepare areas, aseptic technique……**PAGE 15**
12:00	Lunch
1:00	Plant Tissue Culture for Hobbyists, Teachers and "All Plant Lovers" PPT2
2:00	Demo of disinfection and culture of ▸ African violet leaves .....**PAGE 18** ▸ Axillary buds (node sections).....**PAGE 20** ▸ Orchid seed (dry).....**PAGE 21** ▸ Subculture of established cultures .....**PAGE 22**
2:30	Hands-on disinfection and culture of African violet leaves, axillary buds (node sections), orchid seed (dry), and subculture of established culture and work on plant material that you brought
4:00	Discuss problems, trouble shooting, Clean up and return supplies for travel



SAFETY RECOMMENDATIONS



Tissue culture techniques normally used in a scientific laboratory can be dangerous without proper training and/or supervision. Instruction from a qualified plant tissue culture specialist is recommended, and reading the handout and/or the KCK Manual is NECESSARY. Methods included here have been modified to maximize safety of novice tissue culturists. Material Safety Data Sheets (MSDS) provide information on the safe handling of chemicals. An MSDS (in PDF format) for each chemical involved here is located on the MSDS CD. Read about each chemical that is unfamiliar to you before you start working with it. Follow safety recommendations.

- Bleach solutions will discolor clothing and can be harmful to the skin and eyes. Wear protective clothing including gloves, goggles, apron, and shoes. **Plastic aprons are provided; some goggles are available if you do not wear glasses**.
- Alcohol is flammable. Smoking and open flames should not be permitted in the area. A fire extinguisher and running water should be available.
- Sterile technique must be used to minimize contamination of cultures.
    - A **plastic lined box or plastic "re-cycle type" container** can be used as a clean area. The container should be wiped or sprayed down with 70% alcohol before starting work.
    - All items that are put into the clean area should be sprayed or wiped with 70% alcohol.
    - **Hands** should be washed, and then wiped with 70% alcohol, or an alcohol based hand sanitizer.
    - If you have to sneeze, leave work area immediately.
    - **Long hair** should be tied back to minimize contamination.
    - Tools should be positioned in the clean area to minimize passing hands over sterile areas.
    - The 70% alcohol that is used for dipping instruments (forceps, knives) should be positioned to the far right or far left. **Use fresh alcohol daily**.
    - **Do not use an alcohol burner in a plastic lined "clean box".**
    - **Sterile work surfaces** are needed for cutting plant tissues. A small salad plate or a paper towel, sprayed with 70% alcohol, will provide a sterile surface.
    - Media and water should be processed in a pressure cooker or microwave.
    - Food and drink should be kept away from the tissue culture area.

Supplies included in Basic Kit:

The basic kit is designed to minimize cost of using plant tissue culture in a classroom or home by providing minimal supplies. Included supplies are necessary and can be obtained only through scientific supply companies. A few other supplies are included as a convenience. The picture below shows some of these items.

KCK Manual, DVD and MSDS CD
MS Medium with Vitamins–1 Liter Packet (4)
Agar – 9 gram Packet (4)
PPM-10 ml
BAP powder (100 gm)
NAA powder (100 gm)
KOH solutions (100 ml ea) to dissolve plant growth regulators
Baby food jar caps (50)
pH papers
White plastic Mason jar cover
1 ml syringe
Measuring spoons
Forceps (8 inch)
Transfer pipets (1 ml) Graduated
Smidgen spoons
Other

Materials NOT included:	
Sucrose (table sugar)	Hot pads or silicon tipped tongs
Vinegar for pH adjustment	Baby food jars – 6 ounce
Baking soda for pH adjustment	Mason jars (pint or ½ pint) for sterile water
Distilled water	Kitchen knife
Food coloring	Spray bottles for alcohol
Bleach, dish detergent, isopropyl alcohol (70%)	One liter container (microwavable)
Long handle spoon or Magnetic stir plate with stir bar	Microwave oven or pressure cooker
Plastic wrap, Parafilm or florist tape	Clean box
Paper towels	Goggles, gloves, lab coat, shoes

Supplies included in Workshop/Classroom Ready Kit:

The Workshop/Classroom Ready Kit is designed to include all supplies that are usually provided in a KCK workshop EXCEPT for items that must be purchased locally such as bleach, detergent, isopropyl alcohol, vinegar, distilled water, food coloring, as well as items commonly found from home (such as hot pads) and heavy items such as microwave oven and glass baby food jars. See list below.

KCK Manual, DVD and MSDS CD	pH papers
MS Medium with Vitamins–1 Liter Packet (4)	White plastic Mason jar cover
Agar – 9 gram Packet (4)	1 ml syringe
PPM-10 ml	Baby food jar caps (50)
BAP powder (100 gm)	Forceps (8 inch)/Kitchen knife
NAA powder (100 gm)	Measuring spoons
KOH solutions (100 ml ea) to dissolve plant growth regulators	Smidgen spoons
Transfer pipets (1 ml) Graduated	**Long handled spoon**
1 Liter Microwave Proof Container	**Container for sterile water**
Spray bottle for alcohol	**Container for alcohol soak**
Enfamil container for alcohol instrument soak	**Container for bleach soak**
PVC Cleanbox with Plastic bag (30-33 gallon)	**Plastic wrap/Sealing tape**

Materials NOT included in Workshop Ready Kit:

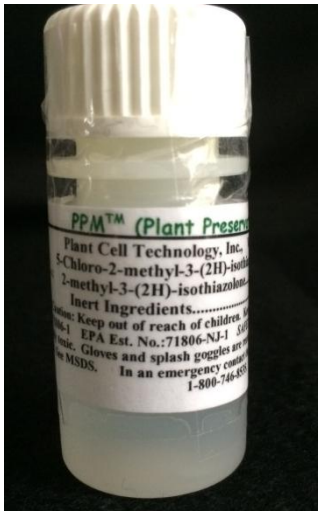
Sucrose (table sugar)	Hot pads or silicon tipped tongs
Vinegar for pH adjustment	Baby food jars – 6 ounce
Baking soda for pH adjustment	Mason jars (pint or ½ pint) for sterile water
Distilled water	Microwave oven or pressure cooker
Food coloring	Paper towels
Bleach, dish detergent, isopropyl alcohol (70%)	Goggles, gloves, lab coat, shoes
Magnetic stir plate (optional)	

Description of Items/Uses

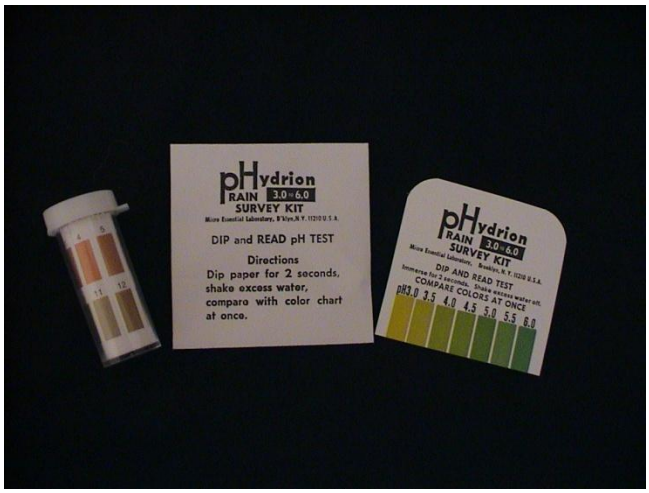
Murashige and Skoog Medium with Vitamins is a basic medium used for culturing many plant species.

It contains macronutrients and micronutrients needed plus vitamins (all pre-weighed) and makes one liter of culture medium

Agar is typically used as a solidifier and provides a physical support for the plant..



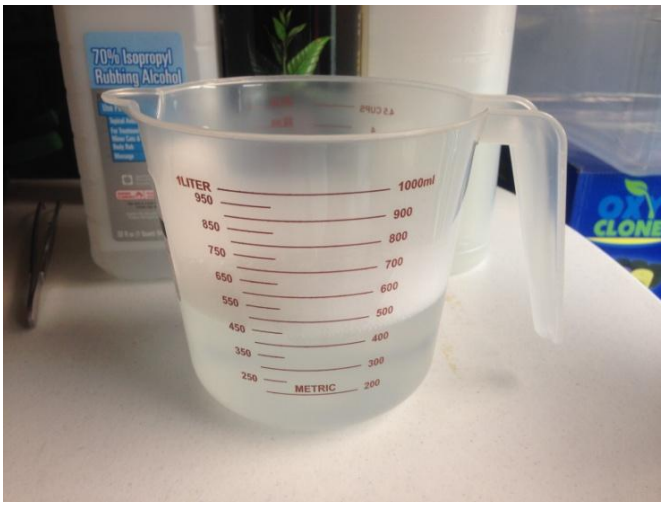
PPM (Plant Preservative Mixture) is a biocide and helps to control contamination.

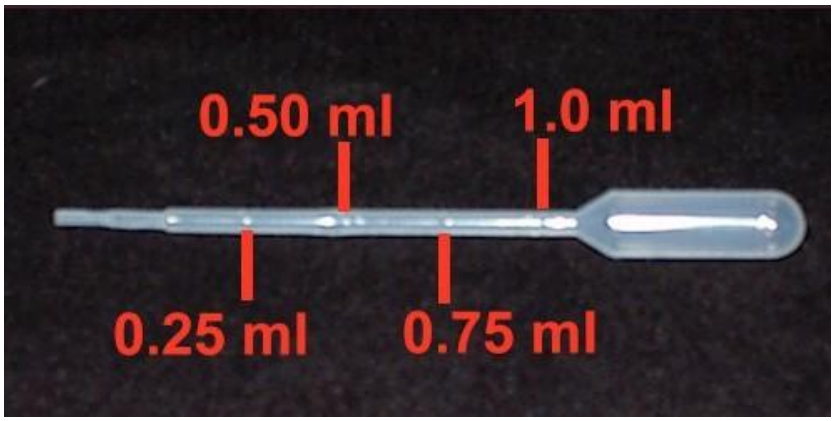


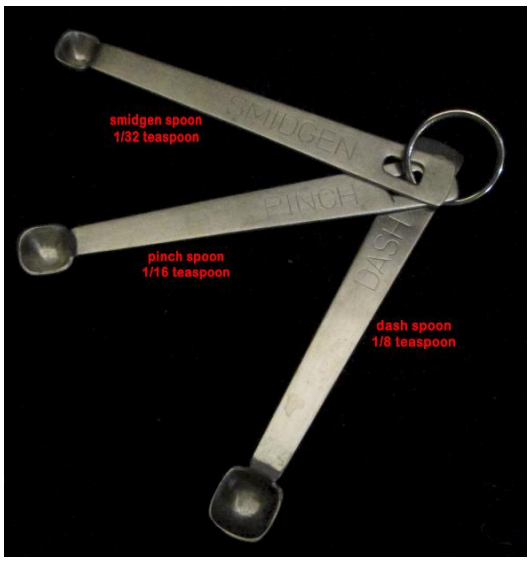
pH papers help to adjust pH which is important in uptake of nutrients.

Plant growth regulators control plant growth – usually shoot induction or root initiation. Plant growth regulators are provided dry (100 mg) with a separate 100 ml of KOH solution (0.1 M).

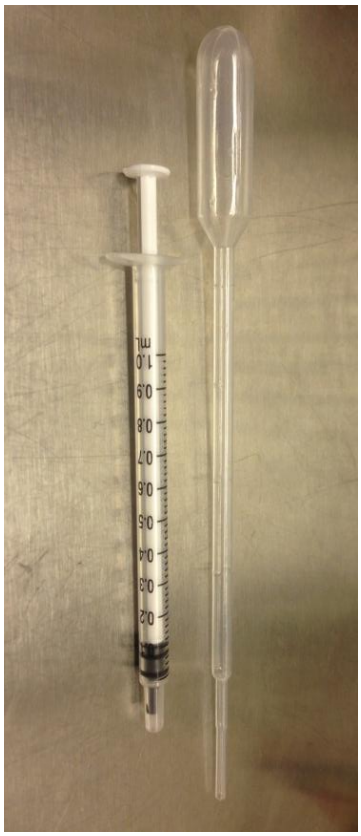
Measuring Devices



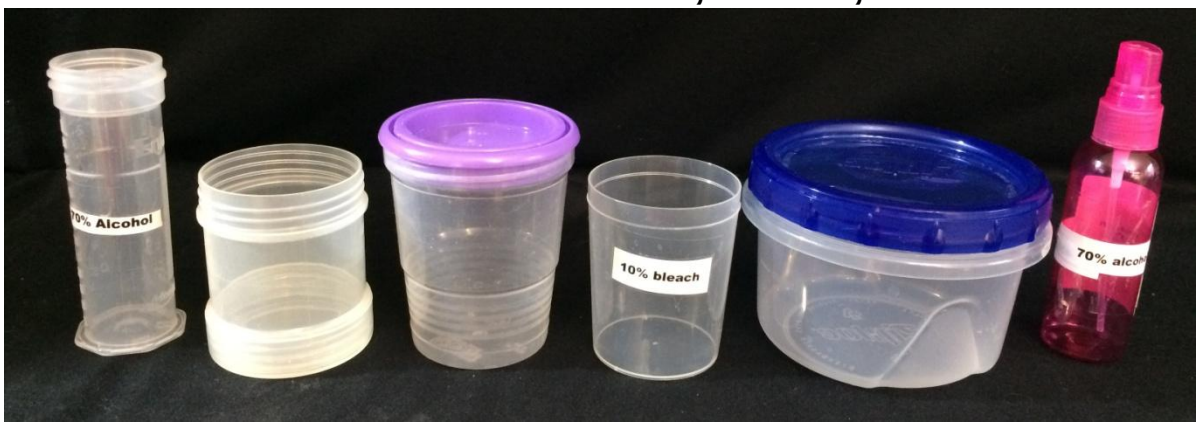




“Smidgen spoons”

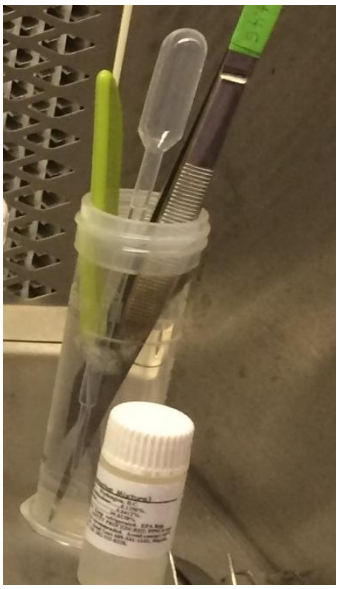


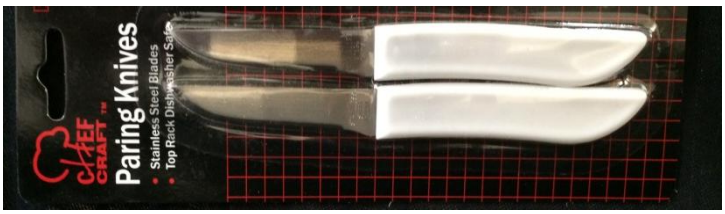
Containers used for water, bleach, alcohol



Different containers can be used for alcohol, bleach and sterile water, but microwave-proof containers must be used to prepare sterile water. Do not put taped labels on the sterile water containers. The tape will not tolerate the heat of the microwave. Do not use metal in a microwave.

Forceps, knives, transfer pipets, etc. need to be soaked in 70% isopropyl alcohol and these containers below work well.



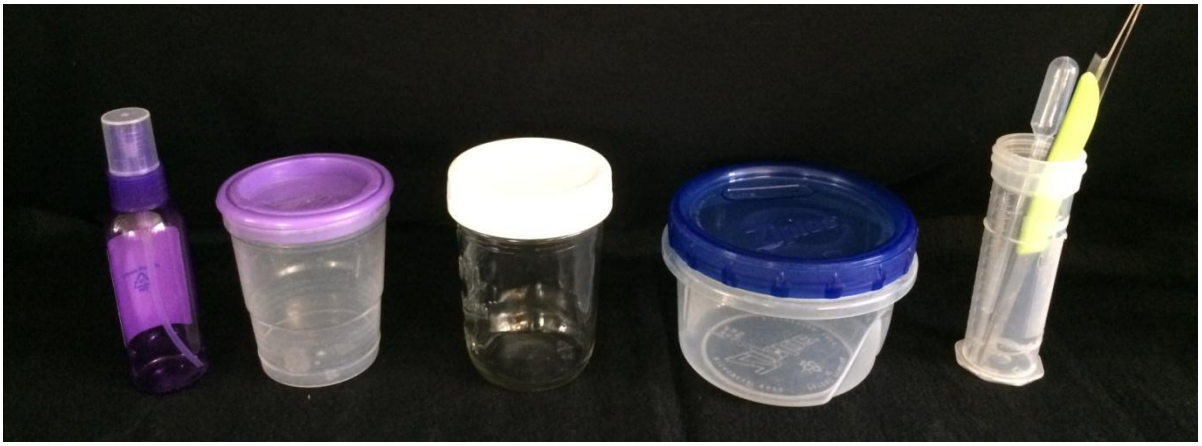


Simple kitchen paring knives are much cheaper and safer than surgical scalpels.

Set-Up in Cleanbox is usually alcohol (left), bleach (center) and sterile water (right) with a taller container for alcohol to soak forceps and knife plus a spray bottle with alcohol. **See pg 17.**

alcohol—bleach—sterile water

alcohol—bleach—sterile water



alcohol—bleach—sterile water

MEDIA PREPARATION

The instructor will work with students to make two kinds of media using household supplies and items provided in today's workshop.

Materials Needed:

• MS Medium With Vitamins packet (1 L) • Sucrose (table sugar) • Agar (powdered) • BAP (100 mg) and 100 ml KOH solution • PPM – 10 ml • vinegar and baking soda (for pH adjustment) • water (distilled or filtered) • food coloring • forceps • Microwave-proof containers for water	• long handled spoon or magnetic stir plate • measuring spoons • "smidgen" spoons • transfer pipettes • pH papers or pH meter • One liter container or microwave beaker • baby food jars with plastic covers • microwave with turntable • Hot pad gloves or silicon tipped tongs

Basic Media Formulas

Axillary Bud or Orchid Seed Medium (Green)

Half Strength MS Medium with No Plant growth regulators:

_____ 1000 ml distilled water
_____ ½ packet MS Medium with Vitamins = ½ teaspoon
_____ 1 ml PPM
_____ 1 tablespoon sugar (about 15 grams)
_____ 2 drops **green** coloring

Prepare as described on following page. Dispense media into baby food jars (2 tablespoons = 30 ml each). Add one level smidgen spoon agar each, OR prepare for pouring: (500 ml media + 11 pinch agar; melt and pour into culture vessel). Process in microwave.

Shoot Inducing Medium (Blue)

MS medium with 1 mg BAP (benzylaminopurine)

_____ 1 packet MS Medium with Vitamins
_____ 1 ml PPM
_____ 1 ml BAP [1 mg/ml] (cytokinin that induces shoot development)
_____ 2 tablespoons sugar
_____ 2 drops **blue** coloring

Prepare as described on following page. Dispense into baby food jars (2 tablespoons = 30 ml each) + one level smidgen spoon agar each. Process in microwave.

Media preparation using a microwave

1. Fill container with about 3 cups (about 750 ml) distilled water. Add the MS Medium, sugar, plant growth regulator and PPM. Mix well with a long handled spoon or mix using magnetic stir plate. ADD ENOUGH WATER TO BRING VOLUME TO almost one LITER.

2. Test the pH of the solution by dipping the edge of a piece of wide **range** (pH 1-14) pH paper into the solution. A pH of 5 to 6 is preferred. Compare the color of the wet pH paper to the pH color chart.

3. If the pH is too low ("acidic"), add a pinch of baking soda to the solution. Mix well and test again.

4. If the pH is too high ("basic"), add a few drops to a few milliliters of vinegar. Stir to mix and test again.

5. Continue this process until the pH is between 5-6.

6. To better adjust the pH to 5.6-5.8, dip the edge of the **narrow range** ("pHydrion Rain Survey Kit pH 3-6") into the solution. Compare the color to the chart.

7. Follow the steps above using vinegar and baking soda to adjust the pH to 5.6 - 5.8. ALTERNATIVELY test the pH using a handheld pH meter. The instructor will demonstrate this.

8. Add 2 tablespoons of liquid medium to each baby food jar using a measuring tablespoon.
9. Add one level "smidgen" spoon of agar to each container. **Note that one level "pinch" spoon of agar is added to 3 tablespoon of media.**

10. Place the polypropylene caps on the jars loosely.
11. Place 4-5 jars in the microwave oven.

12. Microwave for about 3 - 4 minutes. Time will vary with individual microwaves. Watch the liquid and when it starts to boil, continue microwaving for **60 seconds**.
13. While wearing hot pad gloves, or using heat-resistant tongs, remove the jars and sit them on a stable surface. PUSH CAPS ON TIGHTLY.
14. Swirl each jar briefly to mix the media and the agar. **Do not hold the jar by the plastic cap since they can readily come loose.**
15. Look for specs of translucent unmelted agar. If present, microwave for 1 more minute. Remove from microwave, swirl, and allow to cool.

Summary

Mix “ingredients” together as described above

▼

Adjust pH to 5.5 – 5.8

▼

Dispense 2-3 tablespoons liquid media per baby food jar with measuring spoon

▼

Add ONE level smidgen spoon of agar to 30 ml (one tablespoon)
[OR Add ONE level pinch spoon of agar] to 45 ml (2 tablespoon)]

▼

Place cap on jar loosely

▼

Place 4-5 jars in microwave

▼

Microwave baby food jars about 3-4 minutes watching for the first signs of boiling

▼

Count 60 seconds and then stop

▼

CAREFULLY remove jar wearing hot pad glove or using silicon tongs; swirl

▼

Look for clear specks of unmelted agar

▼

Microwave another 30 seconds or until all unmelted agar has disappeared

▼

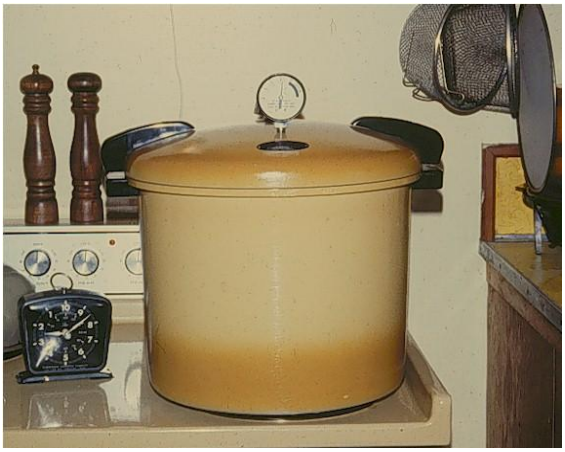
CAREFULLY remove jar wearing hot pad glove and swirl. Press cap to tighten.

▼

Allow to cool.

Media preparation using a PRESSURE COOKER

- Follow instructions above.
- Place plastic cover on baby food jar. Press down for tight fit.
- Place jars in pressure cooker following manufacturer's advice. Jars can be stacked on top of each other. Process 15 minutes at 15 p.s.i. DO NOT LEAVE PRESSURE COOKER WHILE IT IS PROCESSING – PRESSURE FLUCTUATES OFTEN AND NEEDS TO BE MONITORED TO PREVENT ACCIDENTS.



- Allow pressure cooker to cool completely and pressure to go down to ZERO. Open carefully and remove jars using canning tongs or jar holder and place on solid surface..
- While wearing hot pad gloves, or using hot pads, Swirl each jar briefly to mix the media and the agar. Do not hold the jar by the plastic cap since they can readily come loose.

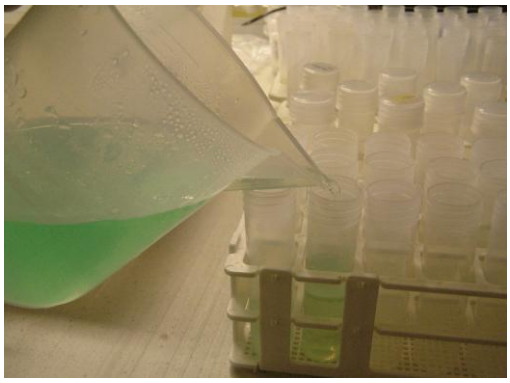
- Allow to cool. Store in plastic container or in ziplock bags on a tray to minimize media drying out.

Pre-melt Media Preparation Method

NOTE: I DON'T SELL THESE TUBES ANYMORE AND I DON'T KNOW IF THIS WORKS WITH GLASS TUBES.

a. Prepare liquid medium as described above.

b. Pour 500 ml into a 1 liter microwave beaker with a spout and handle. **Add 11 level pinch spoons of agar.**



c. Microwave until the solution boils. Stir.

d. Microwave more until all agar is dissolved. No floating clear specs should be left in solution. Do not allow to boil over.

e. Pour about 10 ml into each plastic test tube (about 1 inch). Put screw cap on tube loosely. NOTE: YOU COULD ALSO POUR MEDIA INTO BABY FOOD JARS OR OTHER MICROWAVE-PROOF VESSELS.

f. Place tubes in microwave-proof test tube rack and microwave for 15 seconds. Push OFF button.

g. Microwave 15 seconds more. Watch tubes closely and push OFF button if you see media boil. Continue this for a total of 120 seconds or until you see all tubes begin to gently boil.

h. Remove rack of tubes from microwave. Check media to see if melted. Mix or "rock" the tubes to mix.

i. Cool rack on a slant to increase surface area of medium and to allow moisture to flow down away from plant piece.

Preparing sterile water (used to rinse explants)

Assemble materials:

---Brita filtered water or distilled water

---PPM

---transfer pipette

---microwave proof containers

---one liter container

In the one liter container, add one liter water and 1-2 ml PPM. Mix.

Add about 1-2 inches of water to each microwave-proof container or 100 ml.

Place covers on loosely.

Microwave for about 3 - 4 minutes. Time will vary with individual microwaves. Watch the liquid and when it starts to boil, continue microwaving for **60 seconds**.

While wearing hot pad gloves, or using hot pads, remove the jars and sit them on a stable surface.

Allow to cool. Tighten caps.

Building Cleanboxes

The purpose of the clean area is to limit the number of particles that fall into your tissue culture jar. These airborne particles carry bacteria and fungi, and can kill your plant tissues because they grow faster than the plants.

Break into pairs and assemble a clean box. We have two kinds: PVC, CPVC plus a demo of the “tinker toy” boxes. Use 30-33 gallon clear garbage bag to cover. See page 28 for details on assembly of the PVC cleanbox.

PVC Box

CPVC Box

“Tinker Toy” Box

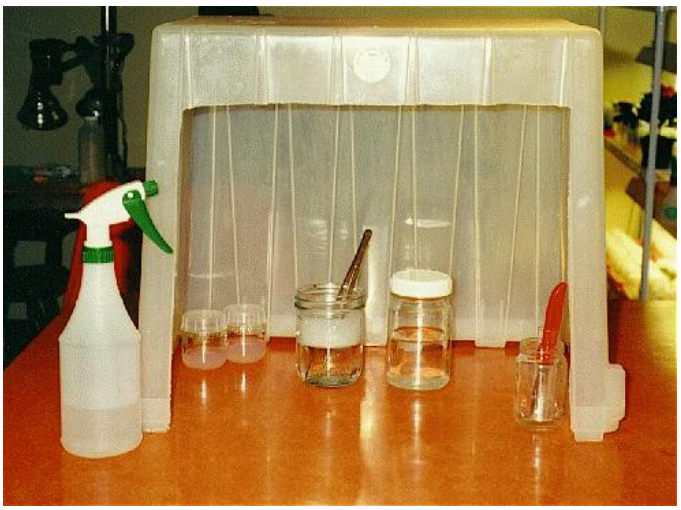
Preparing a clean area

a. The inside of the clean box and the surface of the clean area should be wiped down, or sprayed, with 70% isopropyl alcohol.

b. All items that are put into the clean area (media jars, bleach container, sterile water jar, “dipping” alcohol) need to be wiped down, or sprayed, with 70% alcohol.

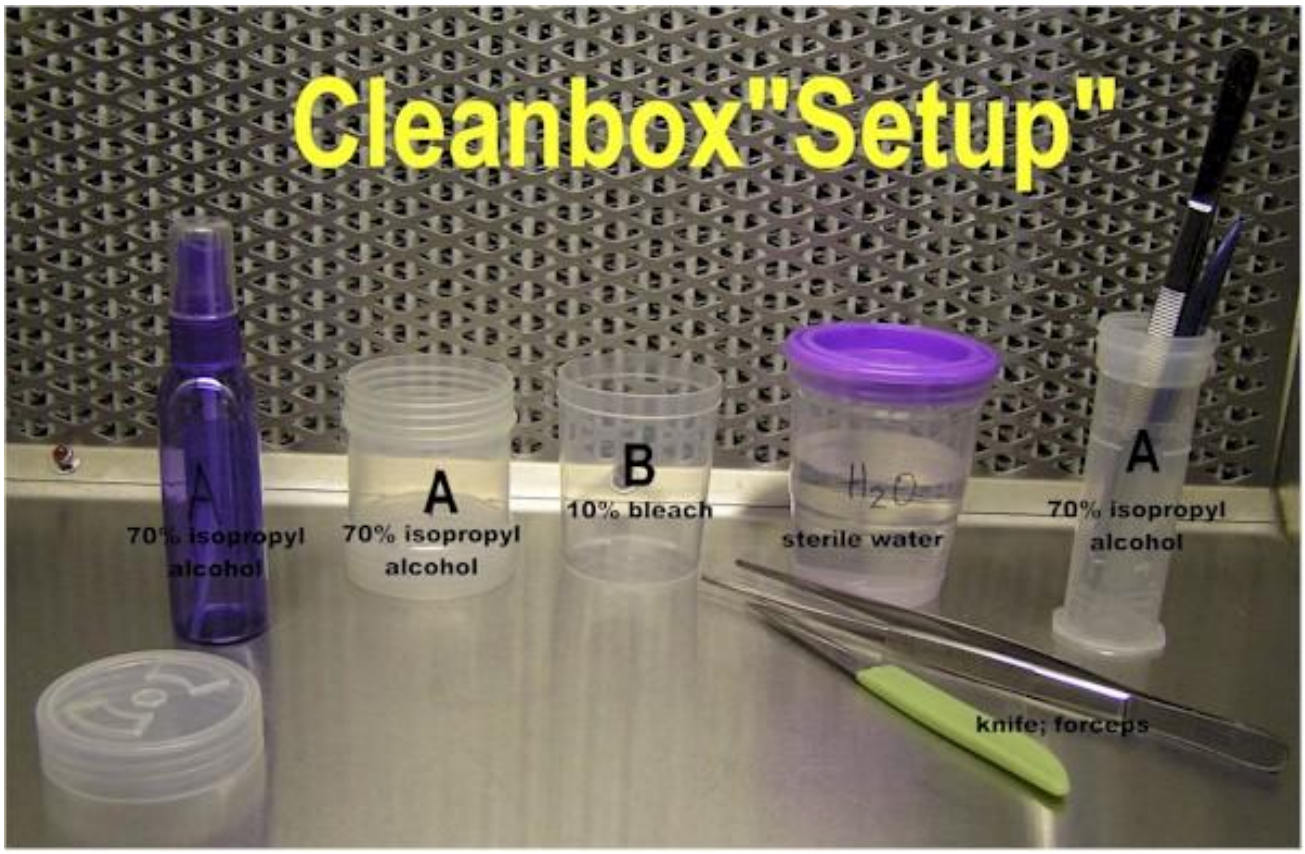
c. Hands should be washed in soap and water for at least 20 seconds, and then wiped with 70% alcohol. Do not use the alcohol on your hands if you have sensitive skin. You can also use the hand sanitizers with ethanol. Vinyl gloves are OK. These need to be sprayed with alcohol.

d. Dip or soak instruments in 70% alcohol. A test tube in a tall baby food jars works well as does a tall “shot glass”, a short bud vase or an olive jar.



Cleanbox “Setup”

Each cleanbox needs these items:



**2 forceps
**2 knives

**alcohol spray bottle
**alcohol container (Enfamil bottle) for soaking forceps and knives

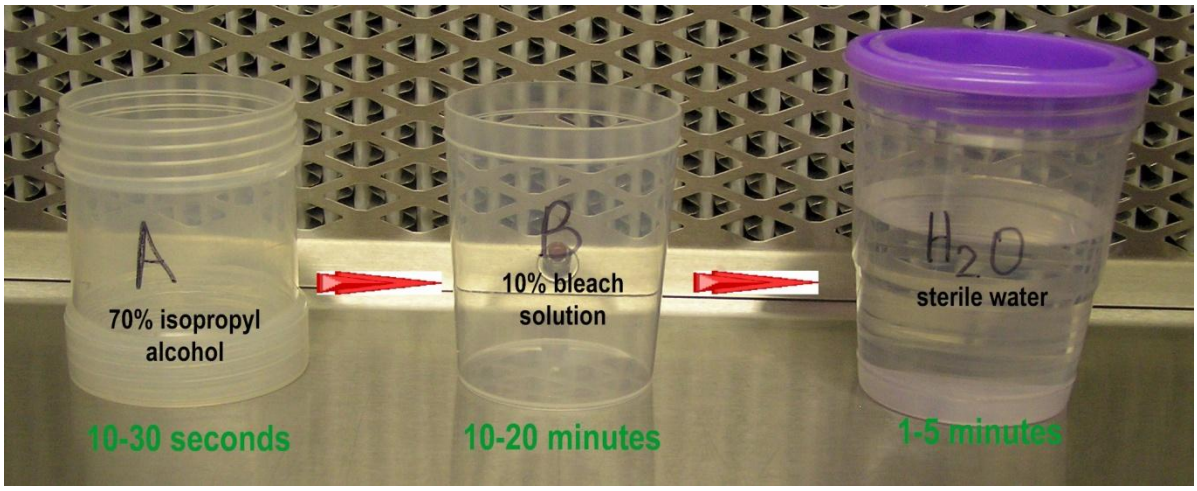
**container for dipping explants in alcohol
**container for bleach solution
**Saran wrap or florist tape for wrapping jars
**sterile water container

African Violet Leaves

Materials needed for the culture of African violet leaves

African violet leaves (1 leaf per person).
African violet medium - BLUE.
70% alcohol (about 1 inch deep) for rinsing leaves.
10% bleach solution (1/3 cup bleach + 3 cups water + a few drops of detergent)
Sterile water for rinsing leaves.
Paper toweling to serve as sterile cutting surface
Forceps and small kitchen knife.
Florists' tape, Saran wrap to wrap jar or tube.

Cleaning the plant material- Gentle Method (see last page for vigorous method)



OUTSIDE OF THE CLEAN AREA:

1. Pick up leaf with a forceps and dip into the 70% isopropyl or ethyl alcohol for a few seconds. This will remove some debris and wax.
2. Place leaf in 10% bleach solution and allow to soak for 10 minutes. Stir occasionally so the solution gets in contact with all of the plant surfaces.

INSIDE THE CLEAN AREA:

3. Move the bottle with leaves to the clean area.
4. Spray area, media bottles, and other containers in the clean area with 70% alcohol.
5. Transfer leaves to sterile water using the forceps that was soaking in the bleach/leaf solution. Allow the leaves to soak for 1-5 minutes in the water.

Culturing the leaves

6. Spray a piece of paper toweling with 70% alcohol.
7. Dip the forceps in 70% alcohol and transfer one leaf to the toweling.
8. Dip the kitchen knife in 70% alcohol and shake off excess alcohol.
9. Holding the petiole end with the forceps, cut the edges of the leaf away. This seems to stimulate shoot growth. Cut off the petiole and then cut the leaf in half.

10. TO MINIMIZE POTENTIAL CONTAMINATION, DO NOT CUT THE EDGES OF THE LEAF. YOU CAN CULTURE A SMALL LEAF WHOLE RATHER THAN CUT IN HALF.
11. Loosen the caps on baby food jars without holding your hands over the cut plant pieces.
12. Dip the forceps in 70% alcohol and shake them to remove excess. Pick up one leaf piece.
13. With your other hand, pick up the cover of the media jar just enough to allow space to place the leaf piece in the jar. The leaf can be right side up, up side down, or sideways. Quickly replace the cover. ***Our goal is to limit the time that the cap is open and the media is exposed to the open air.***

14. Repeat this process for all leaf pieces to be cultured.
15. Wrap florists' tape or saran tape around the outside of the jar. This will help to minimize the debris that gets into the jar and causes contamination of the cultures.
16. Put the cultures in a bright room **out of direct sunlight** or culture on shelves with cool-white fluorescent lights positioned about 9-12 inches from the shelf below Lights should be on 16 hours per day.
17. The leaves should start to swell in 2 - 4 weeks, and small bumps and then leaves will appear on the "mother" leaf's surface. The plant growth regulator, BAP, in the growth medium induces shoots to grow from cells in the leaf.

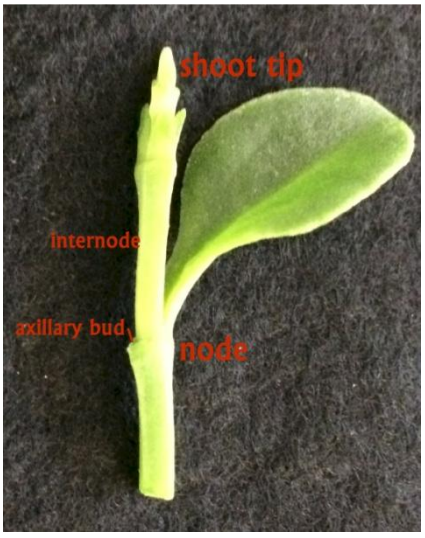
Axillary Bud (Node Section) Culture

Shoots will grow from axillary bud cuttings from many species when cultured on MS medium without plant growth regulators or on other species-specific medium.

A. Cut stems into nodal cuttings with each piece containing an axillary or lateral bud. Stems should be green and vegetative. Remove leaves.

B. Dip the cuttings in 70% alcohol for about 60 seconds.

C. Place in 10% commercial bleach with few drops of dish detergent and soak 10-15 minutes. Stir occasionally using a long forceps or spatula.



D. Transfer explants to a jar containing sterile water and soak for 2-3 minutes to rinse off the bleach.

E. Place one cutting on a sterile plate (wipe off with 70% alcohol).

F. Slice off the ends where the tissue has turned white using a sterile knife (that had been dipped in 70% alcohol).

G. Place in a baby food jar containing the proper medium with no plant growth regulators. The plant piece can be laid horizontally on the medium surface or stuck in the medium ("up and down"). Cap. Seal.

Orchid Seed (Dry)

You will need these items:

- 5% sucrose with a few drops detergent
- 3% hydrogen peroxide
- dry orchid seeds
- transfer pipettes or small knife or forceps
- small tube to hold seeds
- orchid seed germination medium

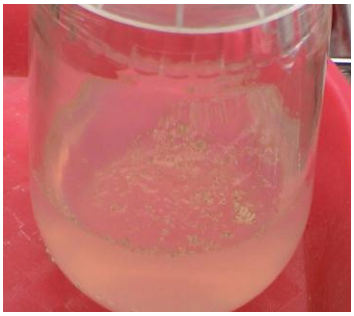
1. Place seeds in small test tube or vial.

2. Add 5% sucrose solution (1 teaspoon sugar in 100 ml water with a few drops detergent) and soak for 12 hours at room temperature. This is not sterile. THIS SHOULD BE DONE THE NIGHT BEFORE THE WORKSHOP. **Note: we often omit this step without any problems**.

3. Remove most of the liquid with a non-sterile transfer pipette

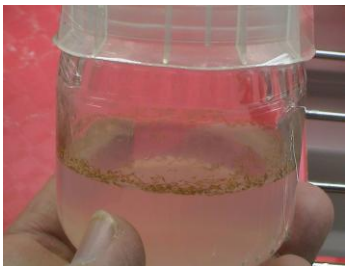
4. Add enough hydrogen peroxide to cover the seeds - about 2-3 ml

5. Place cap on tube and tighten. Shake briefly. Loosen cap slightly and allow to sit for 30 minutes at room temperature.



6. In the clean box, sterilize the transfer pipette with alcohol. Shake to remove most of alcohol. ALTERNATIVELY sterilize the knife by dipping in alcohol. Shake off excess alcohol.

7. Pipette this solution onto orchid seed germination medium. It will resemble a “lake” on top of the medium. Replace baby food jar cap and seal. Label.
   OR
   Use the knife as a spatula and scoop the seeds up and tap into the media jar.



8. Your orchid seeds should grow as seen in these photos. Transfer to fresh medium when seedlings are about an inch tall.

Subculture (transfer) of “plantlets” to fresh medium

The newly developing plantlets will grow better if they are transferred to fresh medium without growth regulators. The growth regulators can inhibit elongation of the shoots and the formation of roots.

This transfer is called “**subculture**”.

- After 4-6 weeks, make fresh medium without plant growth regulators.
- Prepare the clean area as you did before. Wipe off the original culture bottles with alcohol and loosen the caps. Loosen the caps on the fresh media jars.
- Wipe a small plate with alcohol to use as your cutting surface or use a paper towel sprayed with alcohol.
- Dip the forceps in 70% alcohol and carefully remove the plant culture from it’s jar and place on the plate.

- Cut into sections or pull apart plantlets using sterile forceps and knife. Place each small piece or plantlet into fresh medium. Recap and seal.

For further information see:

www.kitchenculturekit.com

Contact Carol Stiff at:

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carolstiff@kitchenculturekit.com
608-302-2750

Vendors:
www.caissonlabs.com
www.phytotechlab.com
www.plantcelltechnology.com

FOR INSTRUCTOR: Media Prepared Before the Workshop [20 Students]:

African Violet Leaf Medium
MS medium with BAP + **blue** color
[2 liter – 44 BFJ – 45 ml each]

Per liter:
_____ 1 packet MS media packet (Caisson Labs)
_____ 1 ml PPM
_____ 1 ml BAP
_____ 2 tablespoons sugar
_____ 2 drop **BLUE** food coloring

Prepare media according to instructions above or in manual: Add ingredients to about 900 ml of water. Mix well. Bring to almost 1 liter. Adjust pH to 5.5. Bring volume to 1 liter.

Dispense 3 tablespoons (45 ml) to each vessel + one level "pinch" spoon of agar.

Process in microwave as described in preceding pages.

Axillary Bud Medium/Subculture Medium
MS medium without plant growth regulators (no food coloring)
[1 liter – 33 BFJ – 30 ml each]

Per liter:
_____ 1 packet MS media packet (Caisson Labs)
_____ 1 ml PPM
_____ 2 tablespoons sugar

Dispense 2 tablespoons (30 ml) per vessel and add one level "smidgen" spoon of agar to each jar. Process in microwave as described in preceding pages.

Orchid Seed Medium
Half Strength MS Medium without plant growth regulators - **GREEN** color
[2 liter – 66 BFJ – 30 ml each)

Per liter:
_____ ½ packet MS medium (= ½ teaspoon) (Caisson Labs)
_____ 1 ml PPM
_____ 1 tablespoon sugar (15 g)
_____ 2 drop **GREEN** food coloring

Prepare as described above. Dispense 2 tablespoons (30 ML) per vessel and add one level "smidgen" spoon of agar to each jar. Process in microwave as described above.

FOR INSTRUCTOR: Media Prepared Before the Workshop [30 Students]:

African Violet Leaf Medium
MS medium with BAP + **blue** color
[2 liter – 66 BFJ – 30 ml each]

Per liter:
_____ 1 packet MS media packet (Caisson Labs)
_____ 1 ml PPM
_____ 1 ml BAP
_____ 2 tablespoons sugar
_____ 2 drop **BLUE** food coloring

Prepare media according to instructions above or in manual: Add ingredients to about 900 ml of water. Mix well. Bring to almost 1 liter. Adjust pH to 5.5. Bring volume to 1 liter.

Dispense 2 tablespoons to each vessel + one level "smidgen" spoon of agar.

Process in microwave as described in preceding pages.

Axillary Bud Medium/Subculture Medium
MS medium without plant growth regulators (no food coloring)
[2 liter – 66 BFJ – 30 ml each]

Per liter:
_____ 1 packet MS media packet (Caisson Labs)
_____ 1 ml PPM
_____ 2 tablespoons sugar

Dispense 2 tablespoons per vessel and add one level "smidgen" spoon of agar to each jar. Process in microwave as described in preceding pages.

Orchid Seed Medium
Half Strength MS Medium without plant growth regulators - **GREEN** color
[2 liter – 66 BFJ – 30 ml each]

Per liter:
_____ ½ packet MS medium (= ½ teaspoon) (Caisson Labs)
_____ 1 ml PPM
_____ 1 tablespoon sugar (15 g)
_____ 3 drop **GREEN** food coloring

Prepare as described above. Dispense 2 tablespoons per vessel and add one level "smidgen" spoon of agar to each jar. Process in microwave as described above.

Quick Method for Mass Media Preparation

Make 5 liters MS Medium with Vitamins
(5 packets + 10 T sucrose + 5 ml PPM)
Adjust pH

↓

Dispense 0MS Media into 44-66 BFJs
(30 ml or 45 ml each)

↓ ↓

Orchid Media (Green)
Pour 1 liter into 2 liter container, add 1000 ml, 1 ml PPM + 4 drops Green food coloring, Adjust pH

↓

Dispense ½ MS Media into 44-66 BFJs (30 ml or 45 ml each)

African Violet Medium (Blue)
To remaining 2 liters, add 2 ml BAP (1 mg/ml) and 4 drops blue food coloring. Adjust pH.

↓

Dispense 1B-MS Media into 44-66 BFJs (30 ml or 45 ml each)

↓

Add agar to all jars; cap jars and process in microwave or autoclave

↓

Swirl, cool, store in plastic boxes or in ziplock bags on trays

Summary of Hands-On Afternoon Activities (Pairs)

EACH **BOX** NEEDS:

**2 forceps
**2 knives
**alcohol spray bottle
**alcohol container (Enfamil bottle) for soaking forceps and knives
container for dipping explants in alcohol (marked ***alcohol*)
container for bleach solution (marked ***bleach*)
**plastic wrap/florist tape for wrapping jars
**pencil

To be handled out in afternoon:
1 sterile water
1 TEST TUBE of orchid seeds

EACH **PERSON** NEEDS:

2 blue baby food jar for African violet leaf
2 green baby food jar for orchid seeds
2 white baby food jar for ax buds

1 African violet leaf (or 2 small ones)
2 node cuttings (with axillary buds)

Before starting culture of plant parts, spray cleanbox down with 70% alcohol as instructed in class. Any containers entering the cleanbox must also be sprayed with alcohol.

1. You will culture one African violet leaf and two axillary bud cuttings. These can be disinfected at the same time:

2. Dip leaf and node sections into 70% alcohol about 30 seconds
3. Transfer these plant pieces to 10% bleach solution.
4. Inside the cleanbox: After 10 minutes in the bleach solution, transfer the plant pieces to sterile water for about 3 minutes.
5. Transfer to culture medium.
6. You will culture orchid seeds – Carol will demonstrate.
7. There may also be time for subculture of existing cultures and establishment of cultures from plants you brought to class. Extra media (blue, white, or green) might be available for these.

Addendum: A More Vigorous Cleaning Method

OUTSIDE OF THE CLEAN AREA

Collect explants. Rinse or soak explants in 3% hydrogen peroxide for several seconds to a minute.

Transfer explants to jar filled with water. Add few drops of dish detergent.

Secure cheesecloth to jar opening with rubber band to prevent explants from leaving the jar. Sit jar under running water for 5-15 minutes (right)**>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>**

ALTERNATIVELY if you don't have running water:

Place leak-proof cover on jar (not cheesecloth), tighten, and shake gently for 10-30 minutes. A Mason jar with metal seal and ring works well and is leak-proof.

Remove explants from water and dip briefly in 70% isopropyl or ethyl alcohol. This will remove some debris and wax.

Place explant in 10% bleach solution. Place cover on jar and shake gently for 10 minutes (right)**>>>**.

10% bleach solution =1/4 cup bleach + 2 1/4 cup water plus few drops dish detergent

Move the bottle with the leaves to the clean area.

INSIDE THE CLEAN AREA

Spray area, media bottles, and other containers in the clean area with 70% alcohol. Transfer leaves to sterile water using the forceps that was soaking in the bleach/leaf solution. Allow the leaves to soak for 1-5 minutes in the water.

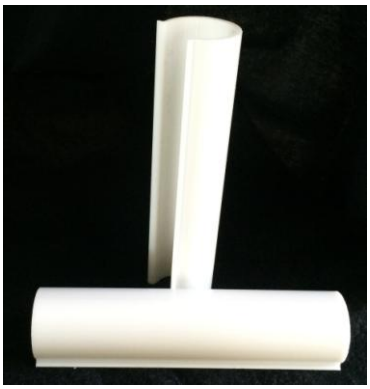
This disinfecting procedure is a little more rigorous and works well with more difficult to clean explants.

Addendum: Steps to Assemble PVC Cleanbox

The Workshop Ready kit comes with ½ PVC pipe and fittings for constructing the cleanbox. The pipes are approximately 20 inches (3) and 15 inches (8) with six 3-way fittings for the corners and two 90-degree fittings for the front plus 4 inch clips (10) to hold the plastic on the structure. A 30-33 gallon plastic recycle bag is included to cover the box.

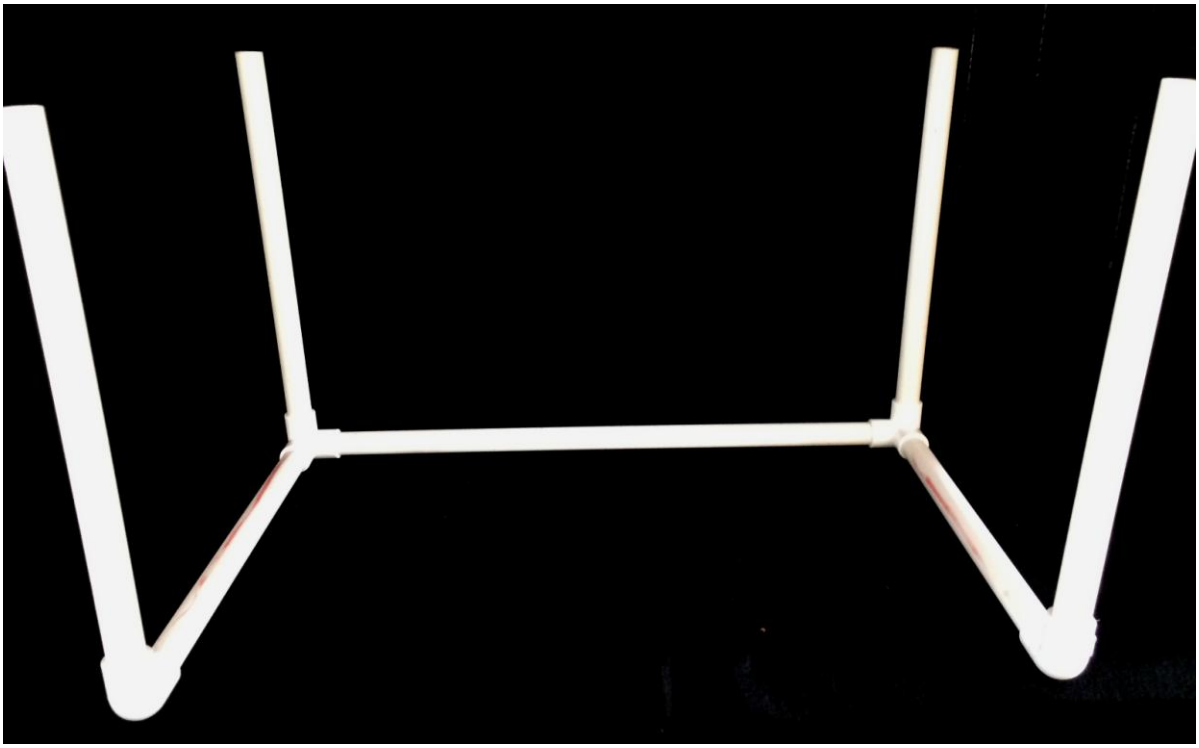
Some prefer a larger box and will use longer pipes (e.g. 36-40 inches) for the width. Plastic drop cloths used for painting can be used for the cover and cut to size as needed. Extra clips may be needed to secure the plastic. (www.amazon.com or www.littlegreenhouse.com)

3-way fitting and 90-degree fitting

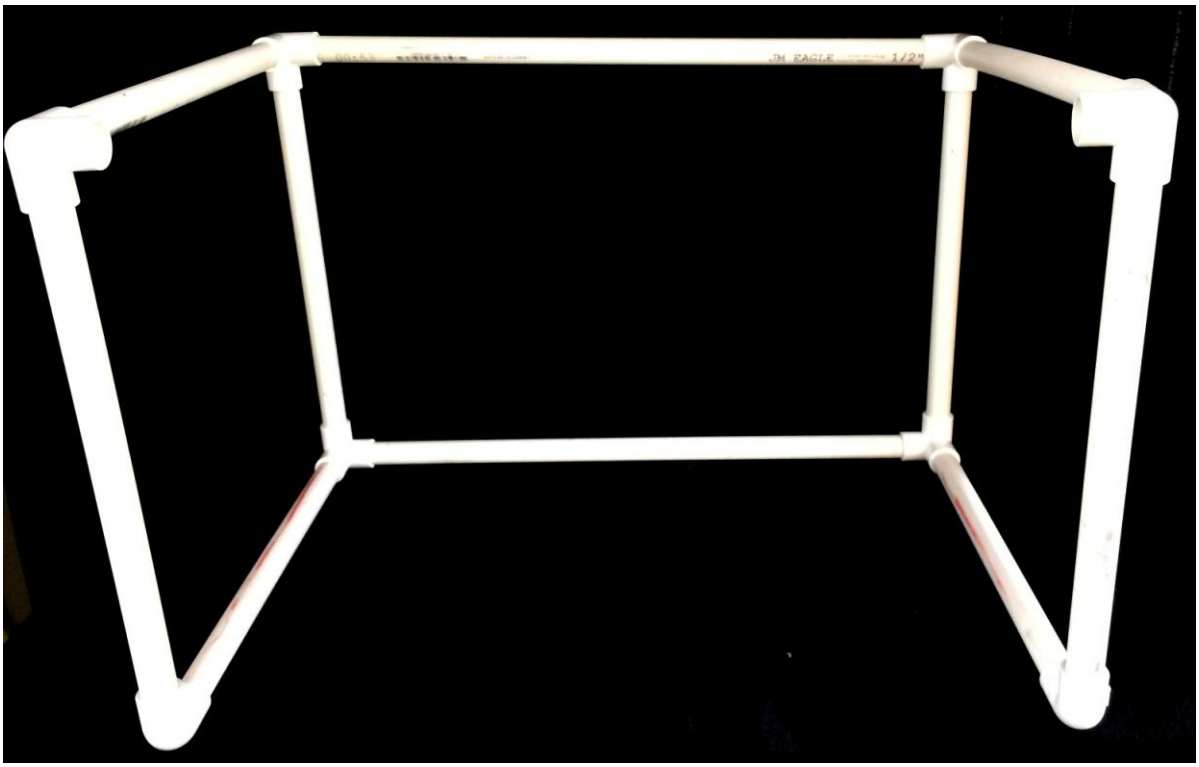


4-inch clip

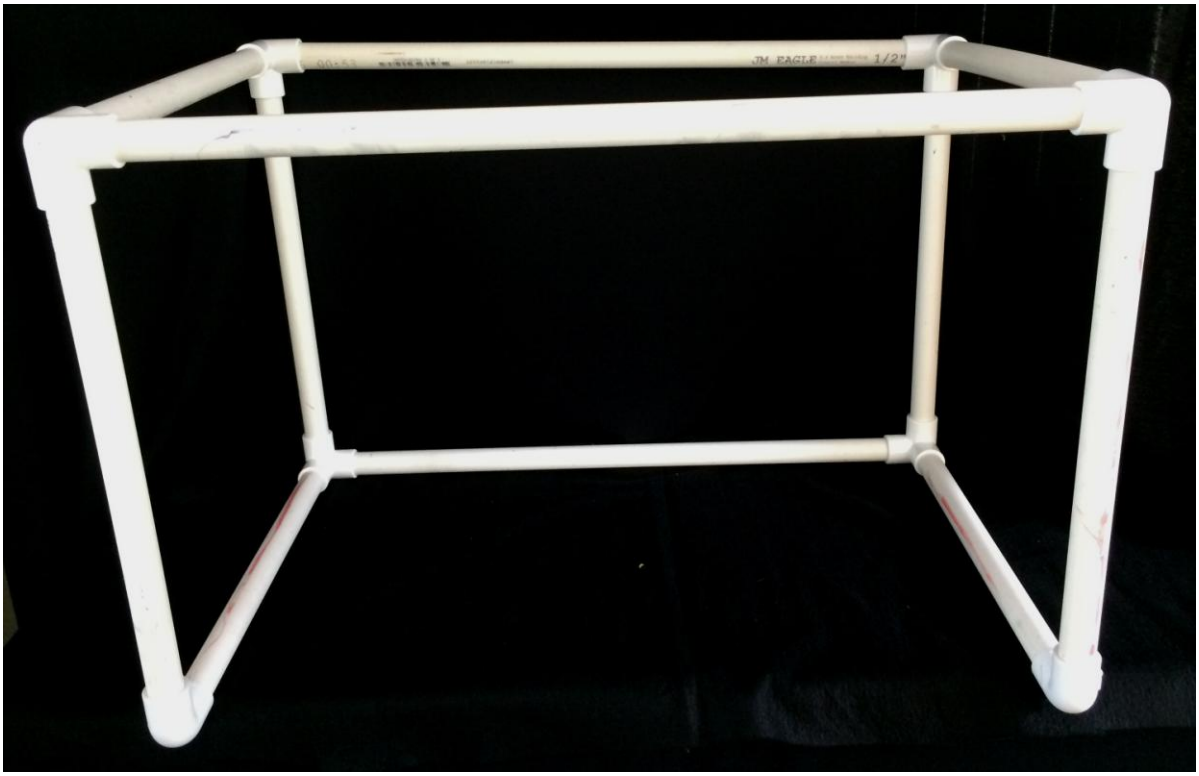
Attach the base as shown. A 20-inch pipe serves for the width while the 15-inch pipes will serve as height and depth of the box. The 3-way fittings are used for the back corners while the 90-degree fittings are used for the front corners.



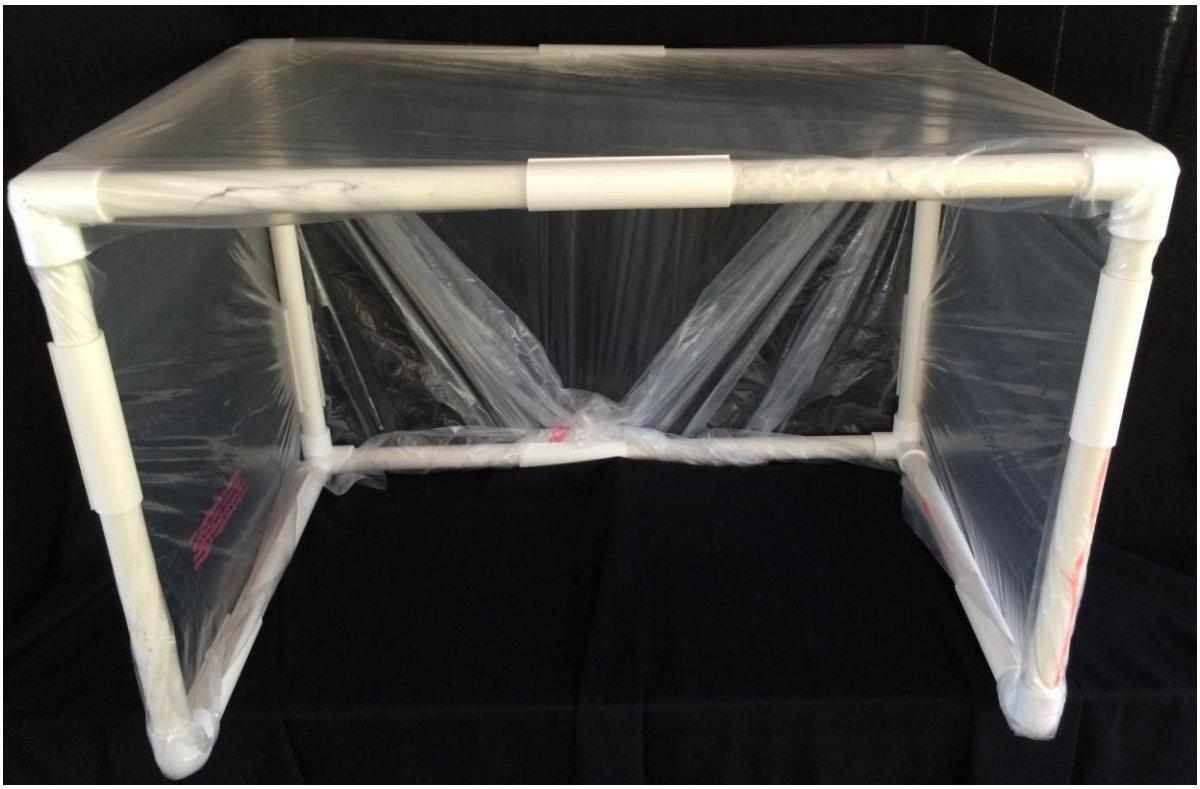
Insert 15-inch pipes at all corners.



Attach 3-way fittings at all corners. Use 20-inch pipe for back and 15-inch pipe for sides.



Finally attach the last 20-inch pipe to the front. Be sure all corners are tight.



Cut one long end of the plastic bag. Drape bag over the box centering it as shown on front pipe and secure with a clip. Secure the front sides next with clips and continue to insure all plastic is secure and no openings exist. This box must provide the dust free environment.