Plant Tissue Culture

An Outreach Project of the Fralin Biotechnology Center

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PLANT TISSUE CULTURE IS A VERY LONG-TERM EFFORT, BECAUSE IT TAKES WEEKS FOR THE CULTURES TO GROW.

HOWEVER, SET-UP BY AN ENTIRE CLASS WILL TAKE ONLY 1 OR 2 CLASS SESSIONS OF 45 MINUTES TO 1.5 HOURS.

DATA COLLECTION (OBSERVATIONS AND MEASUREMENTS) CAN OCCUR DURING CLASS BREAKS.

Dr. Toby Horn taught plant tissue culture to 9th graders from 1985-1994 during the TJHSST rotational technology program, Introduction to Biotechnology, and taught a grade 10-12 Tissue Culture elective course for many years.

In 1989, she published a description of how to make explant cultures of African violets and how to construct the simple tissue culture hood described in this manual (now with pictures) in the January, 1989, issue of the Journal of the Council for Agricultural Science and Technology (text available at http://www.cast-science.org/sfa12.txt scroll to "Lab: Cloning African Violets").

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PLANT TISSUE CULTURE MANUAL

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INTRODUCTION TO PLANT TISSUE CULTURE

Plants have been vegetatively propagated for a very long time. Separating rootstocks, grafting, rooting branches and leaves are all ways to vegetatively propagate plants, by-passing the seed stage . Tissue culture is a newer method that enables more control of environmental factors and has provided the evidence that entire, fertile, seed-producing plants can be cloned from single somatic cells.

Depending on the plant, tissue cultures might be produced from any of these parts:

- Apical meristem
- Flowers
- Ovary
- Pollen
- Stem
- Leaf
- Root, and even
- Seed.

Generally, dicots have been more successfully tissue cultured, but recently monocots like rice have been put into culture. Just this year (1999-2000) rice researchers reported that they could transform rice to have the capability of producing a precursor for vitamin A. Golden colored rice has the potential to reduce blindness in developing nations.

Tissue culture is the method that begins the process for making genetically engineered plants through recombinant DNA technology. However, for decades before recombinant DNA technology started being applied to plants, plant cultures were genetically modified using mutagenic chemicals like colchicine, which often generated larger plants with multiple sets of chromosomes (polyploidy) or by treatment with X-rays to induce mutations via physical breaks in the chromosomes, translocations or changes in the nucleotide sequence (though the mechanisms of mutation at the molecular level were not known at the time these treatments were being used).

Plant tissue cultures can be grown in agar medium or liquid medium. On agar, a solid substrate, the plants can more easily develop roots and shoots.

In suspension culture, the plant material is generally shaken continuously though gently. Bits and pieces break off starting new clumps. These clumps can be pipetted onto an agar surface or the clumps can be coated with a variety of materials. Seed potatoes are actually clumps of suspension cells.

Using plant parts (explants) scientists now study the nutritional and regulator requirements for plant development and cell differentiation and for determining how plants respond to their environment at the molecular level, including how plant cells defend themselves from pathogens (fungi, bacteria, viruses).

Many common food crops and household plants are vegetatively propagated through grafting or tissue culture. Grapes, seedless fruit and roses are generally grafts. Potatoes, African violets, asparagus fern are routinely propagated from tissue cultures. In fact, 95% of the potatoes we eat are generated from “seed” embryos of apical meristem suspension cultures.

Plant Tissue Culture and the Virginia SOL

Plant tissue culture is a set of methods through which students can

- plan and conduct and analyze experiments (Standard BIO.1)
- study the effects of the environment on cell behavior (Standard BIO.5, .8)
- learn about the history of science and collaborations among scientists (Standard BIO.2)
- study hormones and other regulators (Standard BIO.5)
- examine nutritional requirements of cells in culture (Standard BIO.4, .6)
- study health and disease using a model system (Standard BIO.5)
- explore how tissues differentiate (Standard BIO.7)
- and, explore career options.

MATERIALS FOR PLANT TISSUE CULTURE KIT

Please note: Items that do not need to be returned are grayed out.

PLEASE, SAVE ALL BOXES AND BUBBLE BAGS TO REPACK THE ITEMS.

Description	Quantity	Shipping condition	Return condition	Notes
Violet PURCHASE 1/2 classes	1/class			Keep the plant for other expts.
Screwdrivers	2			Please keep the tags on so they can be found.
File folder supports	4 sets			Please disassemble before returning.
Plastic sheeting/tarp	4		Please fold carefully before returning. Wrap an E-flask in each	
Clamps	16			4/hood.
Culture tubes w/ caps	Sufficient for 1/student			Students can bring their exlants home!
Parafilm	Sufficient for 1/student			To seal the opening between cap and tube.
Racks/box/ plastic wrap bags.	1/24 vials			Please return racks and bags so we can ship out more sets.
Long Forceps	4		To carry the leaf+petiole, remove other instruments from alcohol and to plant the leaf piece.	
Curved Forceps	4			For holding the leaf wile you cut it.
Scalpels	4	Please save the wrappers. **DANGER**! Scalpels are **sharp**. Be sure to count at the end of each class. Please repack in the wrappers.		
Bleach PURCHASE	1 pt		Use 200 ml per liter for disinfecting.	
Graduated cylinder, 100 ml	1			For measuring out the bleach.
Magnetic mixer + stirring bar	1 each			For stirring the leaves to be sure the soap and bleach clean the surface hairs.
Large beakers	2			Wrap in a bubble bag.
Liquid detergent	1 tube			Use 2-3 drops per liter for disinfecting.
Rubbing alcohol 70% PURCHASE	1 pt/class		For spraying the inside of the hood and sterilizing the instruments	
Misters	4		To spray the inside of the hood with the alcohol. Before returning, please remove the alcohol and rinse w/water.	
20X150 tube	4			To immerse tools in alcohol.
Erlenmeyer flasks	4			To support the instrument soak tube.
Plastic beakers for waste	4			To collect the sterile water rinses.
Large Petri plates	Varies/units	Use for the rinsing and cutting surface. **Rinse ONLY and return** so they can be UV-sterilized and used again.		
50 ml sterile tubes	**1 tray**	**share w/ all teachers**		**for dispensing volumes of sterile water for rinsing**
Sterile water	1.5 liter per class			Empty all water and return bottles.

Book: Lydiane Kyte, Plants from Test Tubes. **Looseleaf binder: Overheads** of plant, parts, process, hood (in envelope). Teachers manual. Return shipping labels. **Video:** Plant Tissue Culture Methods.

PLEASE RETURN all of these and overheads.

Plant Tissue Culture

A vegetative propagation method that yields many progeny

- A **simple** process that only needs your **full attention** and some **practice**
- A process that starts the production pathway for many, many economically important plant products, including supermarket houseplants and potatoes
- A process that is **rapid** so it IS classroom- and extension- ABLE
- A method that is enabling more nutritious rice and tobacco that produces human proteins for medical use.

Crucial skills:

- **Aseptic handling** to minimize contamination of your cultures by microbes on you or in the air.
- Ability to use forceps and scalpels safely

Helpful outcomes:

- Safe handling of foods and fragile materials
- Attention to detail
- Beautiful plants

Teaching plant tissue culture

Classroom management

Preparing students

- Review or introduce plant anatomy and physiology
- Practice and OBSERVE/correct ASEPTIC handling
- Pass around a specimen to for all students to handle

Logistics

- Limited space? Set up work stations
- Introduce, then run Video while you set up stations
- Limited materials? Each one teach one for replenishing materials
- Attention spans? Circulate to encourage, assess, cajole

Preparation of working materials

- Sterilize culture medium
- Use sterile plates, instruments, and vessels
- Use simple disinfectants in-between
- Gather hood materials (see handout)

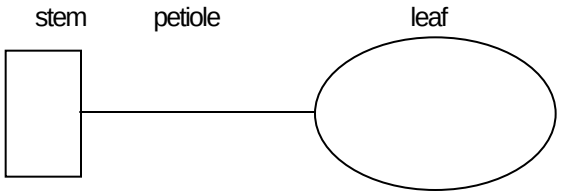
Student involvement

- Practicing aseptic handling (scissors/pen/home work)
- Observing and correcting YOU
- Setting up/taking down hood
- Interdependent teamwork
- Clean-up/Replenishing materials

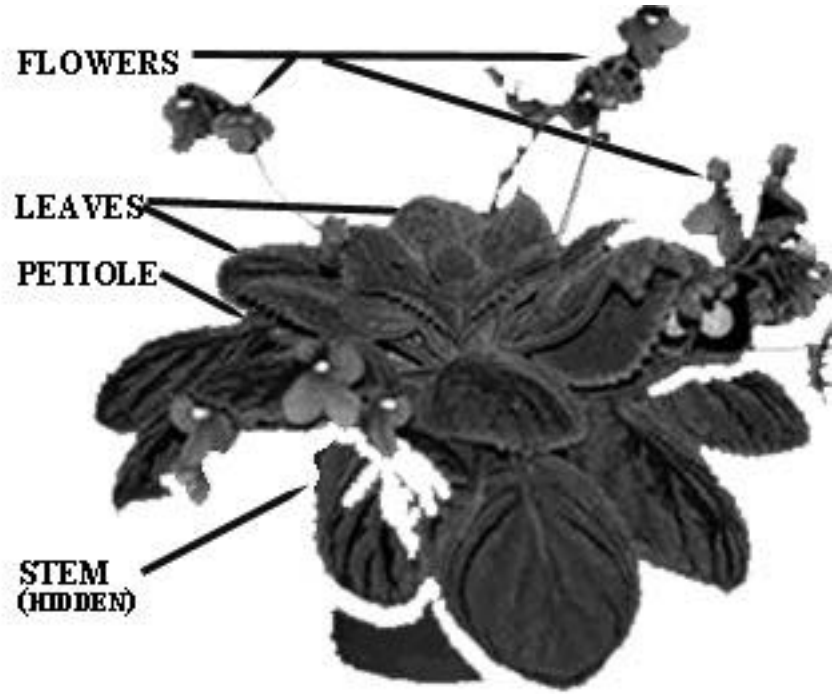
VIOLET diagram

The violet is a generally compact plant.

Leaves are attached to the (hidden) stem through a stemlike PETIOLE



Use the PETIOLE to transport the leaf. Cut the petiole away before cutting the leaf to make the explants. Scientists culture petiole sections, but in inexperienced hands the petiole is a major source of contamination.



Aseptic Technique

Remember: YOU may be the main contaminant of the Plant Tissue Culture
Aseptic technique minimizes --- but does not guarantee --- that your cultures will stay free of contaminating microorganisms

1. Spray the inside of the hood at least 10 minutes before you start to use it, and between teams.

2. Wash your hands thoroughly with soap and water.

3. Prepare your work area --- loosen caps, know where your tools and vessels are, etc.

4. While you are working, stay INSIDE the line of safety bound by the lower bar of the hood support.

5. Be sure that you don't pass your hands or sleeves over open vessels or working ends of tools. Place or move materials out of the way accordingly.

6. Discard all wrappers in regular waste EXCEPT keep the scalpel wrapper.

7. Discard all liquid waste in the waste container.

8. Keep calm while you work as quickly as possible.

9. Rinse with tap water only any items that need to be returned. At the end of the day, rinse all tools to remove the rubbing alcohol.

10. Wash your hands thoroughly when you are done.

Tissue culture is a simple but exacting process. Workers MUST remember that **invisible microbes** are on us and surround us. By working **aseptically,** you can minimize contamination of tissue cultures. A simple hood like the one described here helps to shield your workspace from microbes in the air. You, the worker, contribute to the aseptic method by being observant and compliant about not passing hands or arms over uncovered materials, etc.

Aseptic Handling for Plant Tissue Culture

1. **Use rubbing alcohol full strength from the supermarket (70%) in a clearly marked plant misting bottle to spray the inside surfaces.**
2. **Wash your hands thoroughly.**
3. **Use sterilized or disinfested materials.**
4. **Handle ALL materials aseptically.**
5. **Work somewhat slowly to keep the air relatively still in the area of the hood.**
6. **Do not talk or chat while you work.**
7. **Do not remove any plant material you want to keep unless it is covered with a sterile cover.**

Note that the first time this method is used you can expect that MOST cultures WILL get contaminated within the first 2 weeks. However, students will quickly remember lapses in aseptic handling and by the second try they WILL be successful in having a minimum level of contamination by bacteria or mold.

Strategies to prepare students to be aseptic

1. **Pass scissors with good manners vs. pass scissors aseptically --- holding scissors far from the cutting end and with your hands only on the handles.**
2. **Open a pen that has a cap by holding the pen in the non-writing hand. Pull the cap off w/ the pinkie finger you use for writing, then, hold the cap in the same pinky finger AND write with the pen. (will be hard, but writing is NOT an aseptic process).**
3. **Homework to open jars and bottles aseptically (including toothpaste tubes!).**
4. **Homework to observe where and when aseptic handling can minimize contamination of foods:**

- **Close milk containers as soon as you pour the liquid.**
- **Develop a listing of items that might be nutritious for mold and microbes (e.g., bread, milk, cheese, meat).**

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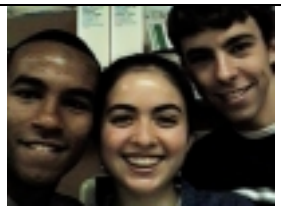
A Home-Grown Plant Tissue Culture Hood that Works!

How-To Build a Simple but Effective Temporary Tissue Culture Hood

Students enjoy working with plants. To be successful however, they need to be aware of where their hands are as they work, concentrate on aseptic handling, and be careful with sharp scalpels.

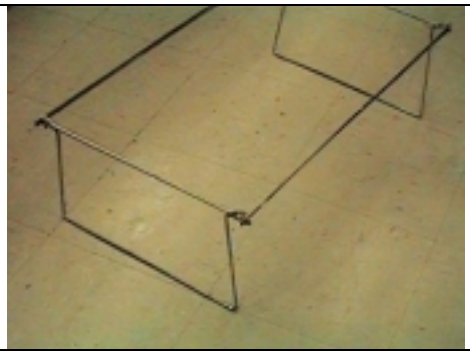
Practice and diligence count!

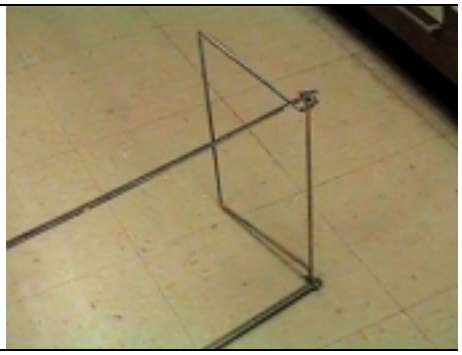


Materials

Screwdrivers

Legal size file folder support



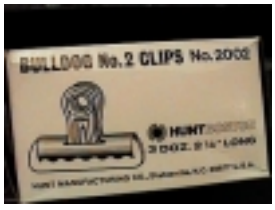


Plastic sheeting (2 mil thickness)

- Smooth out the sheeting and fold in half with the fold at the front.
- Drape over the file folder support. Leave some extending over the file wire forming a short curtain.

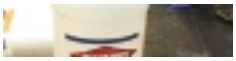
Binder or Bear Clips

Use to hold the plastic sheeting taut over the file folder support frame.



70% Rubbing Alcohol (isopropanol)

from the supermarket or pharmacy



Sprayer-Mister for plants

Use to spray the alcohol around the inside of hood work-space.

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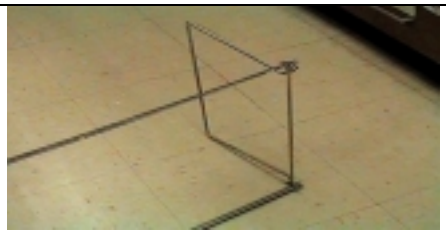
A Home-Grown Plant Tissue Culture Hood that Works!

Constructing the Plant Tissue Culture Hood

Assemble the file folder support parts. Have several screwdrivers available for students to use.

Turn the assembly on its side so the long bars are in FRONT.



Lay the folded side of the sheeting over the long bar of the file folder support.

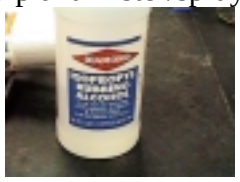
Depending on the layout of your workspace, you may arrange the supports and sheeting differently.

Drape the double sheet so it is even over the file folder support.

The double thickness will help keep the workspace aseptic even if you have pinholes in the sheeting.

Set the fold so it forms a curtain extending about 1/4 the height of the support.

70% rubbing alcohol
+ a plant mister/sprayer



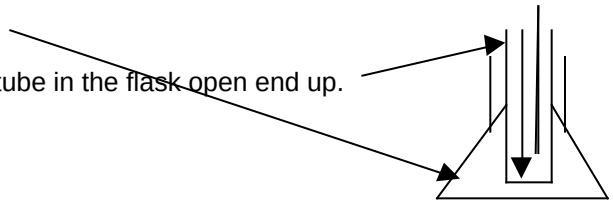
After assembling the unit, spray the inside w/the alcohol. Wait 10 minutes before use.

	Front View	Side View
Clip the sheeting along the sides with "binder" or "bull" clips to hold the sheeting taut over the frame made by the file folder support. Use the clips to make the curtain tight in front, but DO NOT clip along the top. Clip along the sides only.		

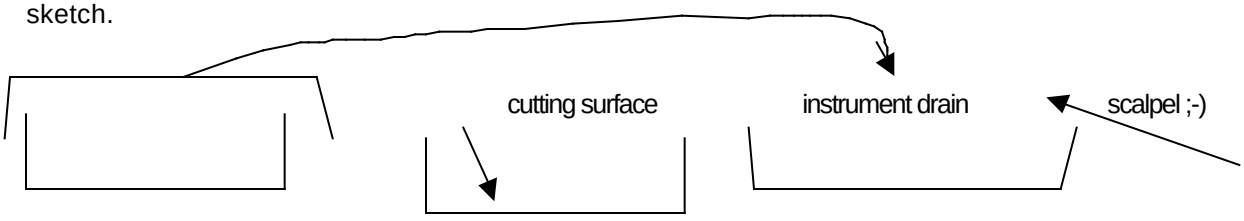
Setting Up the PTC Hood Workspace

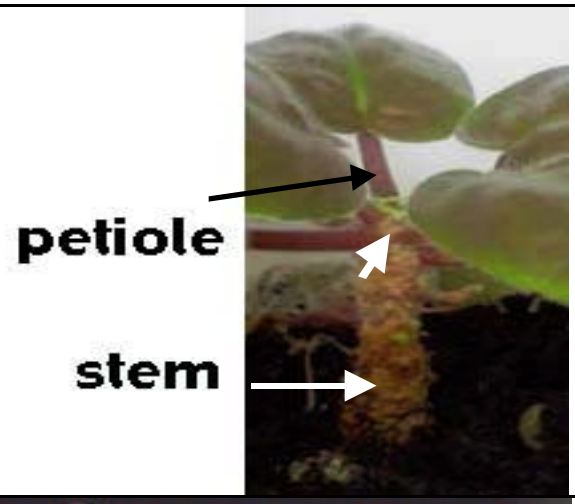
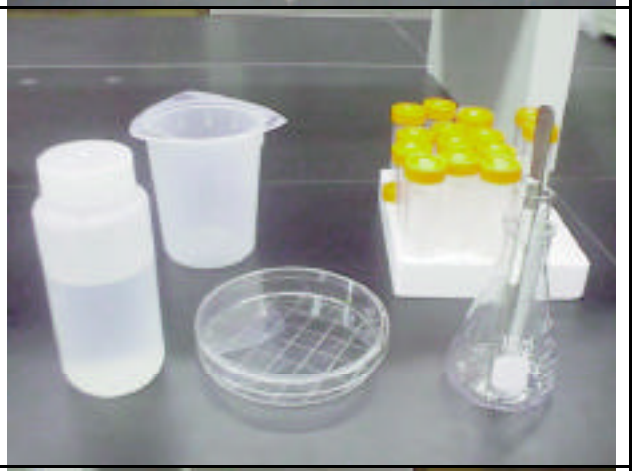
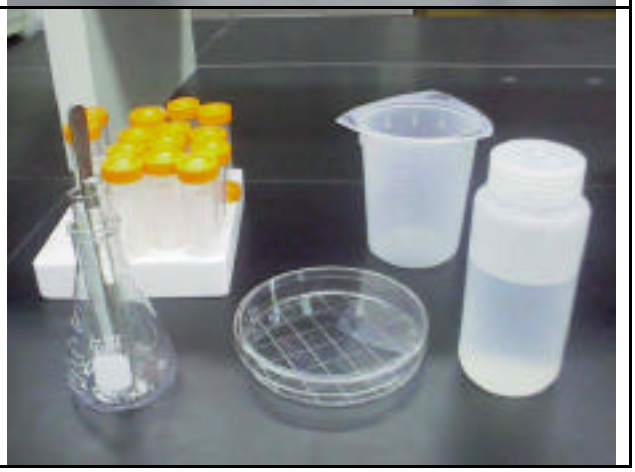
Please outfit the hood in this order:

1. Set the file folder supports so the frame faces forward. If you set it up correctly, one long bar will be on the table in front of you. Tell students to consider this the line of aseptic handling. NOTHING open should go outside this line.
2. Drape the plastic over the top so the fold overhangs the upper bar by about 3-4 inches, forming a "sneeze-protector"
3. Spray the inside of the hood "floor, sides and ceiling, with 70% rubbing alcohol straight from the bottle.
4. Put a large waste beaker (has 3 protrusions) in the back. Students will pour the water rinse waste into the beaker IN the hood.
5. Put an erlenmeyer flask in the hood.
6. Put an empty glass 20X150 culture tube in the flask open end up.
7. Put a scalpel in the tube in the flask
8. Next put in a curved forceps
9. Last put in a large forceps



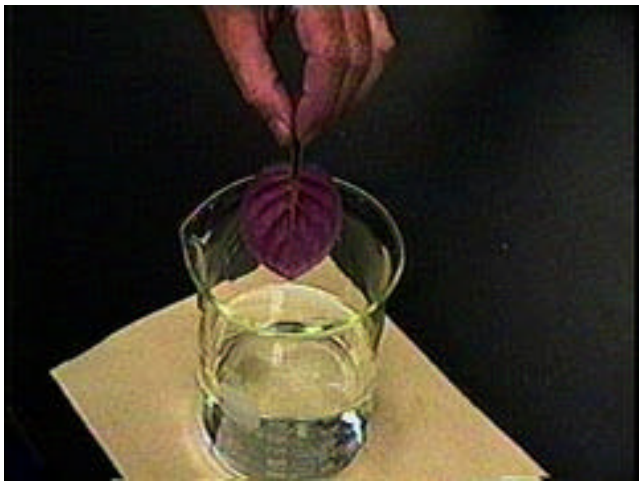
10. Fill the tube to the brim with the rubbing alcohol. This will mean that the scalpel and curved forceps are fully immersed in alcohol.
11. Place in the hood a 50 ml tube w/ small piece of styrofoam to hold it. Use the tube to dispense sterile water into the LARGE Petri Plate. One plate per team of 3 ONLY.
12. Put in a sterile water bottle. Spray the cap rim with some alcohol. Loosen the cap aseptically and WARN students to put the cap bottoms up in back of the hood when they get ready to dispense the water.
13. Spray everything again.
14. REMIND students that the hood is NOT sterile, just relatively aseptic. They must do their cutting on the INSIDE surface of the Petri plate. They can use the rim to set the instruments down to drain.
15. Each team will bring in their 3 culture tubes. The caps should be placed **lid up** like in this Petri plate sketch.

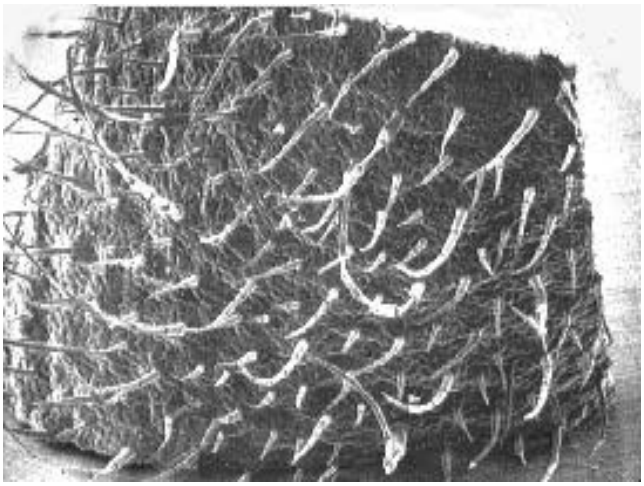


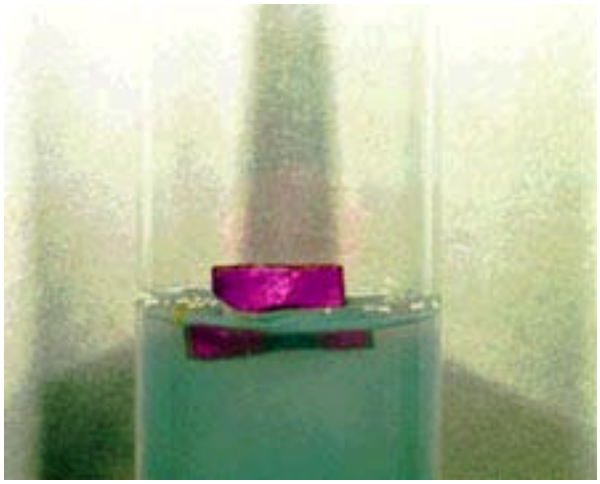
Cut the petiole/stem connection right at the stem (short white arrow). Use the petiole to transport the leaf so you do not injure the leaf. When you are ready to make the leaf fragment explants, cut away the petiole first.	
Add tools to the tube in this order: 1. scalpel 2. small forceps 3. large forceps Fill the tube to the top with 70% rubbing alcohol (isopropanol) to immerse the tools AND handles.	
For a right-handed person: the hood work area should look like this.	
For a left-handed person: the hood work area should look like this.	

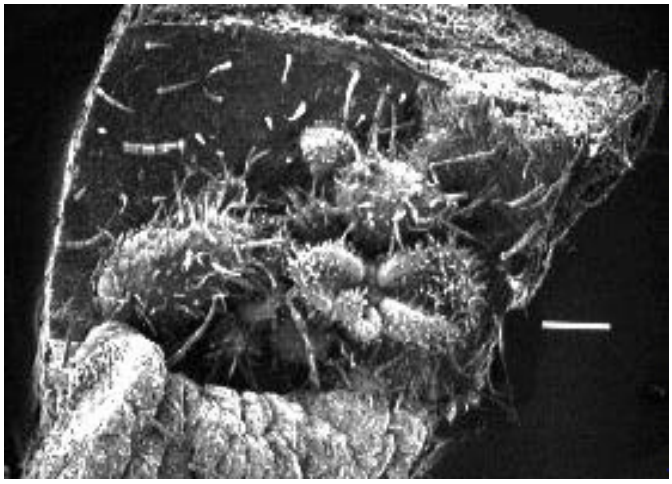
http://www.jmu.edu/biology/biofac/facfro/cloning/cloning.html

Also visit http://www.jmu.edu/biology/pctc/tcstart.htm,
Dr Renfroe's webpage on Getting Started in Plant Tissue Culture









Lesson Plan

1.	Welcome students.
2.	Have students set up PTC hoods.
3.	Pass around a violet leaf (one you might not want to use) for EVERY student to handle.
4.	Have students DRAW/sketch a leaf+petiole on the blank section of the instructions.
5.	Review Aseptic Handling using some fun tricks to engage every student.
6.	Pass out procedure and have students read, then CHOOSE A B and C in their own group.
7.	DEMONSTRATE the procedure, including clean-up.
8.	Assign ABC steps in the procedure so that everyone gets to do all parts.
9.	Show 11 minute video segment while YOU set up the materials in each hood. (4 stations are needed for a 1 h class period. Require students to take notes!
10.	Review the overall cloning process and today's initiation part.
11.	Remind students to ROTATE among: go-fer, monitor and conductor.
12.	Monitor work around the room, encourage students to be careful and aseptic.
13.	COUNT SCALPELS before AND AFTER all work is performed.

When Plant Tissue Culture is Happening in YOUR Classroom, students will be at different stations performing different tasks…

A. **Using DISSECTING** MICROSCOPES	**B.** Using the Cloning Hoods	**C.** Catch-Up and/or Get Started
View the piece of African violet leaf and petiole. **1. Compare the upper and lower surface of the leaf.** **2. Look at the hairs. Count the number of segments (cells) on 10 hairs.** **3. DRAW an enlargement of a representative leaf hair**	Disinfest, Dissect, and Plant the Tissue Teams of 3 work in hood Plant the Aseptic Leaf Cultures	Peruse the books Review the outreach info STUDENTS might look at plant cross - sections or surf the Internet for PTC websites w/applications, methods, and articles

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Developed with James R Johnson, 1985-1992 at TJHSST, Fairfax County Virginia

CLONING AFRICAN VIOLETS

student name
teacher
class period
date

Most scientists do many things over the course of a work day. Each task may be part of a process that can take months, but little progress will be made if only 1 process at a time is done to completion. Folks would get really bored really quickly.

What will happen in your classroom today reflects the activity of a real research lab workplace. The several folks working in the lab each perform a variety of related tasks so that the experiment can have lots of data support and so that the work can proceed productively.

Teamwork is important here. By working well together, each can get more done, and better, than when each is working individually. Help each other and you will benefit.

Cautions! The leaves dry out under bright light. **Scalpels are SHARP!** Work aseptically so that you do not contaminate the leaf.

A.
Observe Leaves with a
DISSECTING MICROSCOPE

View a piece of African violet leaf and petiole.

1. Compare the upper and lower surface of the leaf. Write your comparisons in words on the graph paper. Include features like color, texture, appearance.

2. Look at the hairs. Count the number of segments (cells) on 10 hairs. Describe them as best you can. What common device do they look like?

3. DRAW an enlargement of a representative leaf hair on the graph paper Use 1/4 of the page to draw 1 hair.

4. Make other drawings to remember what the leaf segment looks like.

B.
Use the Plant Tissue Culture Hoods

- Disinfest for 10 minutes
  in 10% bleach + detergent.
- Cut
- Plant
  the Leaf fragment
  (now called the "explant")

Teams of 3 work **IN** each hood.

Remember to:

1. **Work carefully** with the scalpel and forceps.
2. **Help** each other to be **aseptic.**

C.
Review/Search for
INFORMATION

1. Write down questions you might have about biotech careers
2. Write down questions about plant tissue culture (PTC) applications.

Search for answers on the Internet.

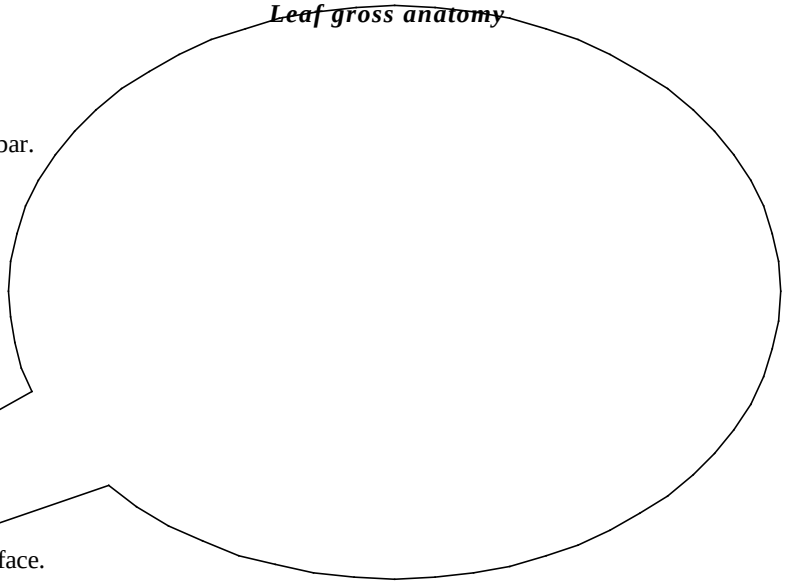
1. Alternatively, STUDENTS might **observe and draw plant cross-sections** or
2. **Conduct an Internet search** for commercial and university web sites w/ PTC applications, methods, and articles.

Cloning African Violets Step-by-Step

1. Disinfest a leaf

- Prepare a large beaker containing 20% bleach, and a few drops of detergent.
- Pinch a small- to medium-sized leaf from the stem so it still has its petiole.
- Drop into the soapy bleach for **10 minutes**. Stir occasionally or use a stir bar.
- While the leaf is disinfesting, set up the hood materials.

Leaf gross anatomy



WASH YOUR HANDS REALLY WELL BEFORE AND AFTER STEP 2.

2. Set up the hood / workspace

- Spray the inside with isopropyl alcohol.
- Set up materials so they can be accessed aseptically. Loosen caps, etc.
- Pour sterile water into the sterile beakers IN the hood **(TEACHER)**.
- Pour 70% isopropanol into the instrument tube that has the forceps sets.
- Remove the shield from a scalpel and immerse in the same instrument tube.
- After at least 5 minutes, set the instruments so they can drain on a sterile surface.

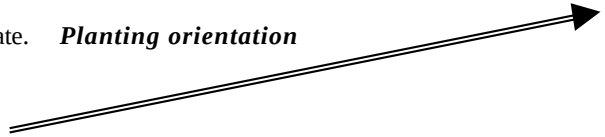
3. Rinse the leaf in 3 changes of water using the very large forceps.

Open the plastic Petri plate and VERY carefully --- and aseptically --- take off the lid and set it open and in the back of the work area.

4. Cut the leaf into plantable parts

- Put the leaf on the bottom part of the plastic Petri plate.
- Cut using the smaller forceps and the scalpel.
- PLANT the leaf part (explant) in the PTC agar tube.
- Plant 1 segment into each tube. Use only 1 tube per person and share a leaf among 2-4 teammates (8 groups per class).
- Replace the instruments in the alcohol and remove the plastic ware that YOUR team used so another team can work at the hood.
- Seal the rim of the cap by stretching Parafilm™ around the tube and cap.
- Label the cap with YOUR name class section and date (including YEAR!)

Planting orientation



5. Clean up

- Rinse out the Petri plate used for the cutting surface to remove any leaf/petiole parts.
- Throw away the wrapper from the Parafilm sheet.

When the first team begins the rinsing process, the next team can start disinfesting their leaf. (Based on nearly 4000 students' experiences.)

Created by Toby M HornOutreachG3:Desktop Folder:~tmhorn:PTC:PTCmanual:VAST violet procedure11/29/00
Developed by TM Horn and JR Johnson at TJHSST. FCPS. VA. 1985-1992

PLANT TISSUE CULTURE

student name
teacher
class period
date

About Tissue Culture in Plant Cultivation

1. What is the usual way that plants propagate?
2. How are potatoes propagated?
3. How are grapes propagated?
4. How are seedless oranges propagated?
5. Why is plant tissue culture a form of cloning?
6. How does a clone compare to a plant grown from seed?
7. Have you ever grown a plant from a cutting? The new plant is a clone. Explain why.
8. What do you think about the observation that many types of plants can be grown from a single, non-seed (somatic or body) cell.
9. What distinctive features can you observe by just LOOKING at a leaf?
10. How does an African violet leaf feel?
11. How does this leaf compare to other leaves you know --- give an example.

About the Leaf Hair Study

1. Provide the number of segments for each of the 10 hairs you counted.
2. List each hair, # of segments
3. Get the average number of segments per hair cell
4. Get the highest and lowest # of segments per hair
5. Compare your values to your partners' values

Explain the differences:

- Include factors like where on the leaf you counted
- Which side of the leaf you counted
- Which leaf you counted
- Order you each observed, collected data

From the data, you can calculate statistics on the variation among leaf hairs. In fruit flies, which also have hairs, interesting genetics can be done that relates the hair appearance with behavior! These are on separate genes, but the cells and functions localize to particular regions in the developing fly!

About Conducting the Tissue Culture

1. Describe how well you think you accomplished being aseptic (not contaminating the work)
2. Describe how well your team worked together.
3. Describe how your team could have improved the effort.
4. How do you think you might do at this with more practice?

About applications of Tissue Culture to Plant Studies and Agriculture

Imagine an application of plant tissue culture you think will benefit society or the environment. Describe how it will be valuable.

PLANT TISSUE CULTURE EQUIPMENT AND SUPPLIES

Description	Classroom Quantity	supplier	#/unit	catno	# unit	cost/per	total	Notes
Violet	1/class	rket/florist/	ea				$ -	Select the middle-sized leaves. Remove WITH the petiole.
							$ -	
Screwdrivers	4	-Mart/hardw	ea		4	$ 1.00	$ 4.00	Please keep the tags on so they can be collected and returned
File folder supports	Varies/unit	ice supply st	4		1	$ 15.00	$ 15.00	Please disassemble before returning
Plastic sheeting/tarp 2 mil th	Varies/unit	ardware stor	1-2		2	$ 5.00	$ 10.00	Please fold carefully before returning
Clamps	4/unit	Office supply	12		1	$ 4.00	$ 4.00	Please clamp together
Culture tubes w/ caps*	Sufficient for	1/student					$ -	Students can bring these home with their explants
25 mmcaps		PGC					$ -	
Racks		PGC		79-3232-30	1	$ 16.70	$ 16.70	
Racks		PGC		79-3232-36	1	$ 16.70	$ 16.70	
Parafilm		PGC	ea	66-1001-01	1	$ 18.89	$ 18.89	Cut into 1/2 square strips
Long Forceps	4	PGC		36-6000-44	4	$ 14.79	$ 59.16	
Curved Forceps	4	PGC		36-5800	4	$ 9.80	$ 39.20	
Scalpels	4	PGC		29-9955-22	10	$ 11.00	$ 110.00	Please save the wrappers. DANGER! These scalpels are sharp! Be sure to count at the end of each cla
Please repack scalpels in the wrappers for safety.							$ -	Rinse all instruments in tapwater and blot dry before putting away. The isopropanol causes the
Bleach	1 pt/gal	Wal-Mart			1	$ 1.50	$ 1.50	instruments to rust.
Liquid detergent (Joy brand is r	1 15 ml tub	Wal-Mart			1	$ 2.50	$ 2.50	Use in the disinfection beaker w/ 10% bleach
Rubbing alcohol 70%	2 pt	Wal-Mart			2	$ 1.00	$ 2.00	
Misters	2	Wal-Mart			2	$ 2.00	$ 4.00	To spray the inside of the hood with the alcohol
Large beaker, plastic	2	Fisher					$ -	Use 1 for the disinfection (10% bleach + 2-3 drops detergent)
							$ -	Use 1 to collect the water rinses if you use the large Petri plate
Magnetic mixer + stirbar	1 each	PGC		43-2904-24		$ 81.00	$ 81.00	Portable (KEEP close watch on this ;-))
25 mm tube closures		PGC	100	79-5320-25	5	$ 43.76	$ 218.80	
25 X150 tube*	4	PGC	500	79-6322-24		$ 95.23	$ 95.23	For immersing the instruments in 70% alcohol and for PTCultures
Plastic beakers	12/class						$ -	Stack and put pack in the box they came in.
Petri plates, 20X150	Varies/units		500		1	$ 80.00	$ 80.00	Use for the cutting surface
Sterile water(distilled water)	2liter per c	Wal-Mart			2	$ 1.00	$ 2.00	Put the cap back on and place in a bubble bag
							$ 780.68	

Plant Tissue Culture References

These books are available in the Virginia Tech Library

Brookhaven National Laboratory. 1956. **Genetics in Plant Breeding.** SB123U47.
3000 copies printed, publication cost, $1.25 in 1956. Even in the 50"s, scientists were transferring genes from one species to another to improve disease resistance for instance, aegilops to wheat!

Highly technical and outdated, but reflects the best in plant breeding of the 1950's. . Article by James Mac Key has an interesting summary of mutation breeding in Europe, with informative (simple) tables to compare the different methods extant up to 1956.
Discussion 1930's colchicine treatment became almost a fad method to make polyploid plants.

George, R.A.T., ed. 1986. **Technical Guideline on Seed Potato Micropropagation and Multiplication.** Food and Agriculture Organization, Rome. callno: SB211P8T421986.

A small manual that describes, in operational detail, how Denmark conducts its potato propagation. Good diagrams and many photographs of the process (B/W). Contains methods for sterilizing, media formulations and making the culture, as well as a timeline for the overall process.

Jensen, N. F. 1988. **Plant Breeding Methodology.** Wiley, NYC. SB123J461988.

Written for the professional, this text goes into great detail about consideration s of factors such as plot size, mechanization of sowing and reaping, and the statistics and interpretation. Features such as basic genetics, crop characteristics, environment, experimental design, project management (including staffing) are all discussed. Just from reading the table of contents you get a glimpse of the interplay of basic science theory, practice and production (agronomy) that students rarely see. The final chapter is "101 ways to enrich your breeding program," a call for remembering to allow genetic diversity plus.

In fact, most seed planted today is really a mixture of different varieties, carefully formulated to provide yield, disease resistance and farming ease.

Kyte, L. **Plants from Test Tubes.** 1987, 1999. Timber Press. Portland, OR. new edition available SB123.6K991987.

The best suited for classrooms and as good a resource as Pierik. Excellent as a basal text for a plant tissue culture course or extended unit.

Has background information on methods, culture media and calculations and examples of tissues to culture. This books also has several chapters on the business of plant tissue culture to introduce students to the factors to consider, in language that is easy to understand, in contrast to the Jensen book (above).

Lawrence, W.J.C. 1968. **Plant Breeding.** St. Martins Press. New York. callno: SB123L391968A.

Plant improvement began at least 9000 years ago, when humans began saving and planting seed. Humans transported seed as they traveled, selected plants that were easier to grow and collect the seed from, and hybridized plants from different regions by gorwing them in the same fields. Through all these routes, humans were vectors of gene transfer.
In the 18th century, plant fanciers collected "sports" which we now call mutants. Domesticated sweet peas were among the first to be documented. Sugar beets were originally grown as fodder. In the 1840's the sugar content was about 7% As new methods for measuring sugar in the beet juice polarigraphically became available, variants that produced more sugar were quickly selected so that, by 1910, the sugar content of beets rose to 16% mutation breeding. Deliberate induced mutations are generated(randomly) by X-rays during the early 1900's. Later, chemical agents were used, after researchers discovered that DNA caries the information that can become mutated. Induced mutations were tricky, most were lethal. In England, X-ray induced varieties include pea and barley and white mustard (1950's and 1960s). In the US, a peanut variety that has better yield and stronger hull than its parent, was introduced in 1959.

Neuffer, M.G., Coe, E.H., Wessler, S.R. 1997 **Mutants of Maize.** CSHL Press. Cold Spring Harbor, NY. callno: SB191M2N391996.

Coffee-table in size, this book has remarkable color pictures, with a good introduction, diagrams and explanations of terms. Like an art museum catalog raisonne'. A comprehensive resource on the anatomy, molecular genetics and physiology of maize, with beautiful microscope and field pictures.

Pierik, R.L.M. 1987. **In Vitro Culture of Higher Plants.** Martinus Nijhoff Publishers. Dordrecht, NL.

An excellent advanced manual for the professional. I used this as the basal text for a 1 semester plant tissue culture course. Although it had no direct information on the plant we were studying, African violets, the information is so thorough that students were able to design their experiments by learning about the ranges of constituent concentrations, formulations, step-by-step procedures.

Written in Dutch English, some of the grammar is noticeably incorrect, but it's an excellent resource nonetheless.

Wang, K., Herrera-Estrella, A. and van Montagu, M., eds. 1995. **Transformation of Plants and Soil Microorganisms.** Cambridge University Press. callno: SB123.57T71995.

Rice (a monocot) was transformed successfully only around 1989-1991.
Highly technical reviews with good tables of methods, cell source and selection criteria.

Plant Tissue Culture on the Internet

Process: Use a major search engine like http://www.altavista.com
Action: Type in "plant tissue culture" (with quotation marks)
Result: Retrieve **6399** links on Wednesday night, September 19, 2000!
Features: First entry is Malaysian! Second is from India. Most are commercial sites.

Look for colleagues
Doug Lundberg's web site at Air Academy HS in Colorado
http://academy.d20.co.edu/kadets/lundberg/index.html

Try Access Excellence
http://www.accessexcellence.org

Visit the US Department of Agriculture
http://www.usda.gov
National Agricultural Library
http://www.nalusda.gov

Even the **National Institutes of Health** has a large plant program, since plant products (aspirin, taxol) have and will continue to provide new drugs.

http://www.nih.gov

http://www.ncbi.nlm.nih.gov

1. Pick PubMed
2. Type in search term(s) (no quotation marks needed)
3. Browse entries (most recently published shows up first)
4. Select Display "citation" and click display
5. Scroll down to MeSH terms (keywords)
6. Use keywords of interest to narrow your search at NCBI and on the major search engine.

Words you don't understand?
1. Click "Books" on the right side of the entry. The same info as before will appear, now with links.
2. Click on a link. You will be connected to a major undergraduate cell biology text.

Plant Tissue Culture Information Projects that use the Internet

Adopt a food or horticulture crop and investigate biotechnology features

Suggest that students go to the National Agricultural Library site www.nalusda.edu

Has the plant been put into cell culture?
Are transgenic varieties available?

Tobacco biotechnology and find data on such as
Plant tissue culture conditions
Conditions for transformation
Tobacco mosaic virus
Biochemistry compared to bacteria, yeast, animal proteins
Tobacco DNA that has been sequence (http://www.ncbi.nlm.nih.gov and pick GenBank) (almost 3200 sequences!)

Plant Tissue Culture using common nutrients

Plant Tissue Culture at home

Hormone effects on plants

Rice, Tobacco, Arabidopsis, Brassica Genomes

VIDEO RESOURCES
AVAILABLE FOR LOAN FROM THE FRALIN BIOTECHNOLOGY CENTER

Part 1 Overview on Plant Tissue Culture Applications
25 minutes, CalState San Luis Obispo VEP

Part 2 Plant Tissue Culture Methods
40 minutes, CalPoly San Luis Obispo VEP, tape 145

Part 1 Overview on Plant Tissue Culture Applications
25 minutes, CalPoly San Luis Obispo VEP

Part 2 Plant Tissue Culture Methods
39 minutes, University of Texas, Lubbock, CEV multimedia, tape 749

Each part 2 tape has a segment on the actual tissue culture process for African violets that is less than 15 minutes in length.

Tapes are available for loan for 1-week periods (with free round-trip shipping).

To request a tape:
contact Ms. Angela Worrell at atawney@vt.edu

or order through the Fralin Biotechnology Center web site at

http://www.biotech.vt.edu
then, select “outreach”
then scroll to “video loan program”