



HTCG Newsletter

No 4, September 2011

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Updates and request for help

Carol Stiff, WA, USA

President and Executive Director, KCET/HTCG

Welcome again to the latest HTCG Newsletter. Our newsletter team headed by Arthur Sale and Toni Annable did a fantastic job with the last issue! I strongly recommend you go back and read it again as it has much valuable information that even I forgot was in it. You can find it at: <http://www.hometissueculture.org/htcgnewsletterMarch2011.pdf>

As stated in previous newsletters and on our website, our mission is to make home tissue culture more accessible and affordable, and part of this is supporting teachers. I am pleased to report that we have supported numerous teachers this year by connecting them with local hobbyists who can help the novice teacher get started with classroom tc, with other vendors who send free samples, and of course, the HTCG sending samples and discounted products. We have also donated a few prizes to some tissue culture related contests and to teacher training workshops. And, if you have ordered from us lately, you should have received some free samples with your order. Hopefully all of this helps promote this wonderful hobby!

The last issue also reviewed who the HTCG is and some history on how "we" came into existence. We asked for volunteers ("site coordinators") to host workshops and/or become instructors. Toni Annable, who has far more business savvy than I, agreed to be our "workshop coordinator" for a few months. We got a good response and had workshops scheduled in Minnesota, Michigan, Texas, Florida and New York. Unfortunately, even with Toni's energy and dedication, we still were not able to get sufficient numbers of registrants for some of these. We have to attribute it to our current economy, the increase in airline costs and our lack of grant money to subsidize the costs. Anyone with excess airline miles to give away, please contact me.

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Toni is now encouraging me to set up online classes which would save money and allow more people access to home tc techniques. We welcome comments and suggestions from anyone on how we could implement this. Note that I hate being on camera and really think the solution is to find more instructors and offer regional workshops. These would cost less due to reduced travel expenses. However, very few have offered to do this. Yes, I am trying to talk Frank into doing this as he is so comfortable in front of the camera but so far, no luck with him either.

Another idea was to have "home tc parties" like the Tupperware parties. Small groups could meet at the home of a local hobbyist and do short demonstrations similar to what we do in the workshops. I need to check with a lawyer and with our insurance company to see how that could be implemented without liability problems.

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Editorial — Issue #4

Arthur Sale, Co-editor, Tasmania, Australia

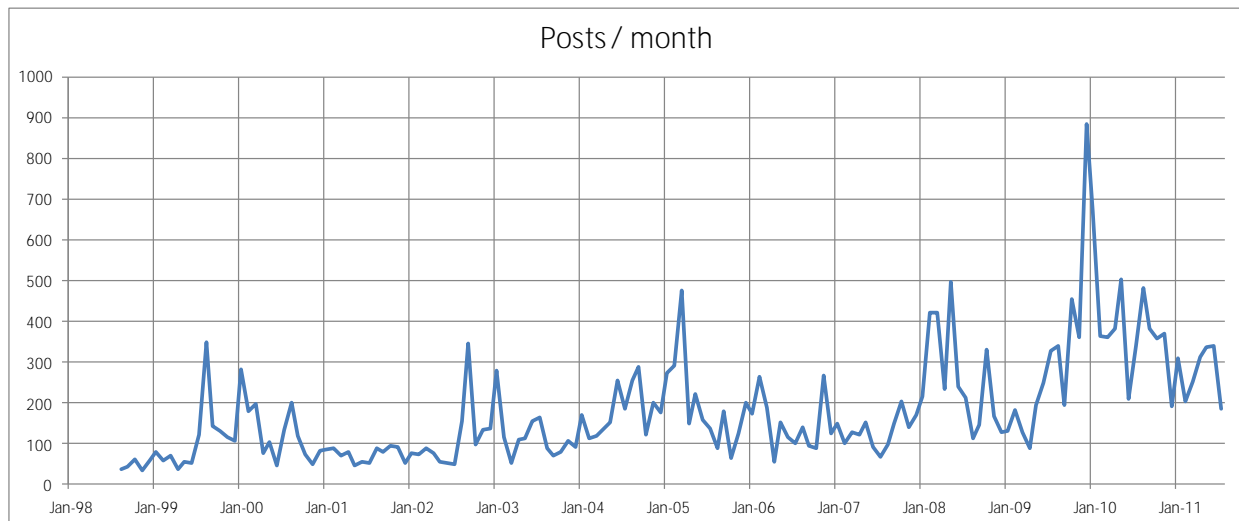
This is the fourth issue of the **HTCG Newsletter**, and the second to be produced under the new co-operative editorship. We plan the next issue to be a *Special Issue* with a Guest Editor who will collect articles with a common theme. If possible, we'd like to do that once a year. If you want to volunteer to be a Guest Editor, please contact someone on the Editorial team.

The Home Tissue Culture Group is a rare thing, to be treasured. The activity of plant tissue culture is itself one of the world's rarest hobbies (maybe sharing this distinction with another of my activities: artisan cheese-making), and the completely collaborative nature of the enterprise should be obvious to

all. How otherwise would we be able to grow so many different plants? Or rescue species from extinction? And why is the HTCG listserv so active?

And yet, we still have trouble filling an issue of the Newsletter. We ask for contributions and very few (if any) come. We go out and target possible authors and 'heavy' them to contribute, and we get a few articles (very few). The role of a Newsletter Editor is not an easy one. Sure, all the editors have a defined role, whether it be in copy-editing, layout, copyright, teaching, or whatever, but one thing binds us all – where do we get the articles to fill the next Newsletter? It is not easy.

The HTCG listserv has 3118 members and it produced 64 posts (questions and answers) since my last visit to the site. All those members must think the posts are worth reading as otherwise they would unsubscribe. Those that post and get replies are even more happy generally with the help they get. So how do you react if I say that anyone who benefits from the HTCG listserv (even by passive reading) thereby acquires a obligation to give something back to the community?



One way to give something back to the HTCG community is to write an article for the Newsletter. So what about starting it now? It can be a description of your lab, what you aim to do, what you have done, about your favourite plant, a protocol or procedure, how to build some useful equipment from scratch, pictures of your environment or the workshop you just went to, etc. I can keep on going, but basically the topic can be anything that you would find useful or interesting to read. You know what interests you. Why not write about it? The editors will help you with the text, and you'll get a permanent record of how you contributed to the progress of tissue culture.

I write with optimism that plant tissue culturists are generous and sharing. Carol, Toni and I look forward to reading your draft article and developing it with you.

Continued from Page 1

For now I will try to make another DVD that basically goes through what we do in the workshops including the slide presentations and then video/images of the media preparation, cleanbox setup, culture initiation from African violet leaves, node sections and orchid seeds and subculture. I think once people learn the simple basics, then they can go on to more advanced techniques.

Please send your suggestions to us (Toni, Frank, Arthur, me). If you are a grant writer and want to share your skills with HTCG, contact us. If you are interested in sitting in on a board meeting, contact me. We meet virtually via Skype (no video) so you will need a microphone and a Skype account.

Frank's in Thailand

Sojikarn Sataporn ('Mook'), Thailand & Frank Tromble, USA

Frank sent us this delightful picture of a pop-up book made by tissue culture students in Thailand. There is more over the page, so please keep reading...



Pop-up book

All pictures © Copyright Frank Tromble, 2011.

Continued from p3: Frank's in Thailand

November 25th 2009 was a decisive day in having plant tissue culture included in the science class at the School in Nakorn Nayok in Thailand. A teacher, whose nickname is Mook, which in Thai means Pearl, had relocated to Nakorn Nayok and applied for a job teaching science. Mook taught plant tissue culture for the last 20 years and wanted to teach it at her new location. The school lacked equipment and funds to have such a course so they improvised as best as they could, but in order to make it a successful course they needed a little help.

Working with what you have: the infamous “Bee Hive” steamer (below left) was their autoclave. It's not pressurized but after several hours of heating and cooling during a 24 hour period it would sterilize the media. Sterilizing utensils? No problem with Mook's ingenuity and less than \$1 in supplies (below right).



Mook and Tummy (the school principal) sought help for the plant tissue culture project from Her Royal Highness Princess Maha Chakri Sirindhorn. Since I was involved with their website, *Botany in School*, helping Mook and the students try to accomplish plant tissue culture with what they had to work with, Mook asked me to help with a presentation to the Princess. Now I don't speak Thai at all and they don't speak English very well. I thought this should be interesting. Thank goodness for Google translation.

The Princess is well educated having spent her whole life studying which, she continues to this day. The Princess' academic accomplishments are quite long. She speaks eight languages, plays several musical instruments, and has received MAs in both Oriental Epigraphy and Pali-Sanskrit. She received a Doctor of Philosophy of Education Development and to our benefit, has studied about plant tissue culture in her Biology classes. Thais don't call her the Princess of Technology for nothing. In other words you have to know what you are talking about before you present a project to the Princess. Nothing like a little pressure.

On the next page is the first formal presentation to Her Royal Highness Princess Maha Chakri Sirindhorn, about the plant tissue culture project for the school in Nakorn Nayok. The presentation on November 25th 2009 was given at the Chulachomklao Royal Military Academy by Tummy (school principal), Teacher Mook and students of Nakorn Nayok. If the Princess liked what she saw she would give the project her blessing and funds will be provided to make plant tissue culture a course at the school.



November 25th was a day filled with anxiety and nerves were on edge hoping everything would go smoothly. It was Thanksgiving Day in the US and I was hoping we would be thankful for the events in Thailand as well.

The Princess has a way of making everyone feel at ease even though there is always an entourage of assistants traveling with her. Behind the title, the necessary formality of being one of the most well-known public figures in Thailand, the Princess is a pretty cool lady, down to earth, genuine and kind. No wonder everyone in Thailand loves her. She approved the project for plant tissue culture to be taught at the School in Nakorn Nayok. The image right says a lot about the Princess and who she is as a person. Always a smile and always so interested in helping promote education.





In 2010 the students demonstrate their knowledge of plant tissue culture to other schools. Tummy and Mook laid out the roadmap for sharing their knowledge via the web and greatly expand the project.

Another very important thing happened in 2010. Shawn Mayes read our #2 edition of the Home Tissue Culture Newsletter and sees a section about a plant tissue project in Thailand schools. Shawn is an author and expert on *Nepenthes* (pitcher plants). Shawn works with scientists from around the world and conducts many expeditions to search for new species. Hoping to pass his knowledge along to the students in Thailand, Shawn contacted Dr. Carol Stiff and the rest is history. Shawn now teaches the students at Nakorn Nayok and other schools in Thailand, plus gives lectures at universities about the importance of conservation and scientific knowledge of this wonder plant, the *Nepenthes*. Shawn's work has added momentum and synergy in promoting plant tissue culture and plant science in the schools.





Thanks to Shawn Mayes, Her Royal Highness Princess Maha Chakri Sirindhorn now has a *Nepenthes* growing in her garden. This isn't just a photo op. The Princess really put the *Nepenthes* in her garden.

Our newsletters reach far and wide. It's surprising where they end up and who reads them. Shawn Mayes teaches the students about the propagation of *Nepenthes*. The students love it. They are always flocking around Shawn and now have a new appreciation for the need to conserve this valuable native Thailand plant.



It's spreading. Twelve other Thai schools now have plant tissue culture as part of their science course. The schools share their knowledge via the website *Botany in School* <http://botanyschool.ning.com>. On the previous page is a picture of a class at the Rajaprajanugroh School in Thailand which has just started to learn about tissue culturing of *Nepenthes*. It looks like the little TC'ers are multiplying as fast as the *Nepenthes*.

Will it continue? Is it worth the investment? Are students getting anything out of it? The project recently came up for review to decide whether it should continue to be supported. The powers that be said yes and increased the funds for the project next year. Below is a poster congratulating Tummy and Mook for receiving the funds needed to continue their project.



What will 2012 bring?

Students are using plant tissue culture as a way to pass the entry exam and be accepted into a University. Getting accepted to a university in Thailand is what kids in the US would call a "Free Ride". The government pays 100% of the costs. It opens up opportunities to bright kids who would not have the chance to attend. You just have to convince the committee that you are qualified, not an easy thing to do. They ask questions. Lots of them during the interview. Three students have passed the exam and seven more are expected to pass having demonstrated plant tissue culture and having demonstrated their knowledge of it to the examination committee. The students entering the universities will be studying various disciplines, Botany, Medical Sciences and other scientific fields with some doing research. Who knows where their new paths will lead them?

Plant tissue culture has opened many doors for many people and has had a real impact on many lives. It brings people together from across the globe who would never have had the opportunity to meet. If you have a minute, stop by the *Botany in School* website to say hello: <http://botanyschool.ning.com> The teachers and students can translate English into Thai using Google Translator and will be more than delighted to hear from you and you never know what a chance meeting will bring.

Tissue Culture from the Kitchen to the Classroom

Amy Thielman, Agriculture Instructor, Chilton High School, Chilton, USA

As a veteran high school agriculture teacher, I have tried numerous tissue culture kits out there for my Greenhouse class, and each one failed every time. I had all but given up on doing tissue culture in my classroom-it was hard to justify spending the time on the lesson and not have any success to show for it. I was torn, as an agriculture teacher, not only do I want students to understand the content, but I strive to give them the hands on experience to apply their learning. Then I ran across one of Carol Stiff's workshops being offered at the Wisconsin Association of Agriculture Educator's professional development conference. I signed up immediately. It was the best decision I could have made. Not only did I learn a wealth of information, but I gained essential resources that helped me create a lab setting where students could find success doing tissue culture. Carol's *Tissue Culture in the Kitchen* program has made all the difference in the world!

For starters, the agar is an easy solution that we can make in class and there is ample opportunity to explore with different hormones and recipes. In addition, all the materials are very economical-which is crucial in today's tight school budgets. I've used Carol's program for the past two years now and am happy to say that **every** one of my classes has had success doing tissue culture! Students are now able to see how tissue culture works as they eagerly watch for the callous formation and even 'compete' with each other to see who has the best sterile technique. We run on a block schedule, so the class period has since ended, but I still have students stop by to see how their cultures are doing. They are eager to transplant the subcultures to fresh agar. When community members stop in, everyone is curious about our cultures-I've even had the science teacher stop by since he heard the students talking about our lab. I'm eager to share your website with anyone who asks. Thank you goes out to Carol for taking the time to do workshops and share her wealth of knowledge to make tissue culture labs fun! I couldn't ask for a better resource.



Two students at work disinfesting

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Why plants are immortal

Arthur Sale -- Tasmania, Australia

Have you ever given a thought to the miracle of being able to clone plants in tissue culture? When cloning animals is so difficult that even experts only partly succeed? Or why plant cuttings and grafts work at all? Apart from sea-stars and a few other exceptions, a cut-off leg does not grow into a new animal nor does the old animal grow a new leg. Plants manage both tricks with ease.

Of course no single plant is immortal—it gets old and ages, but plants manage to get along quite well without sex if they have to. Stands of genetically identical trees have been dated to at least 10,000 years, and vegetative propagation of cultivars (like apples and many nursery plants) is so common as to not excite even transient interest. Try that with any animal!

All living things on Earth have evolved from the same sources, including plants. Plants and animals diverged about 1300 Mya (million years ago). All plants are *eukaryotes* (organisms with cell walls). Each plant cell contains a nucleus and several other organelles like the chloroplasts that turn light into chemical energy and the mitochondria that are the cell's power stations. The nucleus of a plant cell is our target in this article and it contains a collection of DNA (deoxyribonucleic acid – fragment pictured right).

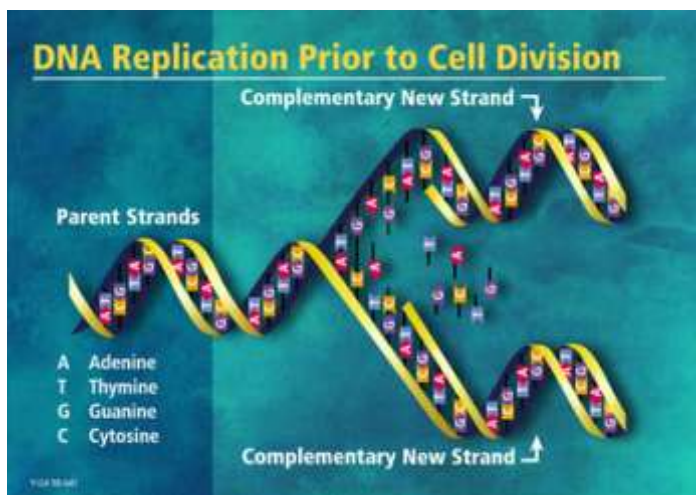


The DNA in the nucleus is not just a jumble, but is organized into several long 'strings' (called *chromosomes*) of DNA. Humans have 46 chromosomes (2x23); the intensively studied model plant *Arabidopsis thaliana* has 10 chromosomes. *A. thaliana* is a weed (left), but it grows and seeds fast, which is good for research. Each chromosome consists of a helix (or twist) of twin-stranded DNA; each strand consists of a sequence of the four *bases* aka *nucleotides* A, T, C and G (Adenine, Thymine, Cytosine and Guanine), and the two strings match up A↔T, C↔G. A good way to think of a DNA strand is as containing a set of recipes (known as *genes*) for making a bunch of proteins. Each protein does something in the organism, and there are ways of turning genes on and off, or throttling them back.

If a plant is to grow, its cells have to grow bigger (limited scope here) or it has to grow more cells. This is what it does by duplicating each chromosome in the nucleus of a cell at intervals, then separating them into matched sets, and breaking the cell into two each with half of the chromosomes. Duplicating is achieved (a **very** rough description) by unzipping the DNA helix and making up a new matching half for each strand. This whole process is called *mitosis*.

And so we come to the key problem: the ends of the chromosomes. The unzipping protein cannot get accurately to the very ends, so that with every cell division, a bit of the ends are lost. If this resulted in losing a gene, the plant would be in real trouble and the cell would probably die. Eventually the plant would too. It happens to animals. So what do they do? The chromosomes have special bits at each end called *telomeres* (Greek for 'far end') which don't contain genes. In most plants (*Aspergales* which includes onions have a slightly different sequence) the telomere sequence is a long repeated sequence (two strands shown):

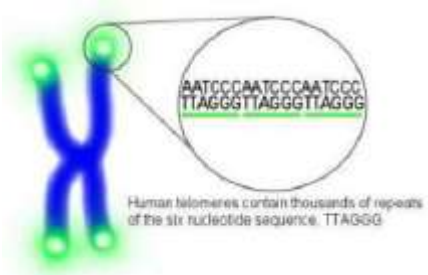
... TTTAGGG TTTAGGG TTTAGGG...
... AAATCCC AAATCCC AAATCCC...



In animals (like mammals and insects) the gradual shortening of the telomeres seems to be implicated in the lifespan of the animal. If they get too short, we are back with the first problem and the death of the cells and eventually the animal. The length of the telomere is like a programmed limit to the number of times a cell can replicate. When an animal is cloned, like Dolly the sheep, it gets the shortened telomeres of the clone parent, and accordingly it starts off life already as 'cellularly old' as the clone parent, and dies sooner than a normal lamb (for Dolly).

Plants have a repair protein called *telomerase*, which looks at the telomeres and adds more repeats on to them if needed. (Animals also have telomerase, but they don't use it much except in reproduction. The human telomere repeat is TTAGGG – just one base different from plants.) This is one reason why some trees can live to incredible ages and sizes – their telomeres are repaired.

Plants do age – you cannot mistake a young conifer *Pinus radiata* with an old one. The young one is a nice conical shape, the old tree is scraggly. However the ageing mechanism is different and slower. Another example is the existence of natural clones. There is a normally long-lived Tasmanian tree called **Huon Pine** (*Lagarostrobus franklinii*), with a one hectare stand of 100 trees on Mount Read which are *all* male. There are *no* females nearer than 20km and *all* the trees in the stand are *identical* clones. The best explanation is that they have been isolated without a female for 10,500 years, and have just reproduced by natural vegetative propagation, probably by natural layering. This is a long time for the DNA of an original seed to survive! Immortal? Pretty much.



The other half of what I wanted to write is that plants have a lot of what would be called 'stem cells' in animals. Being unable to move, mostly, plants have to deal with predators differently than running away. They use poisons and bad tastes often, but they also have ways of dealing with being munched. If a growing tip of a plant is munched off, the bit that is left is deprived of the hormones (auxins) that the tip produced. These hormones suppress nearby shoot growth that might compete. Without the tip, new shoots grow, and the plant recovers.

The message here is that whereas in animals, most cells eventually decide that they are going to be liver cells, or muscle cells, or whatever, and settle down to turn off all the other options (this is called *differentiation*, and is very efficient), in plants many cells keep their options flexible as long as possible. In technical terms they retain *totipotency* (being able to be any sort of cell). They turn off the unwanted proteins via active hormonal control rather than by more-or-less permanent methylation. Many cells in plants are totipotent, though younger parts of plants may be more flexible than older ones. For example old conifer branches often do not re-sprout after pruning whereas young ones do. Palms are really tough to propagate except at the single apical growing tip. Apple trees are much more forgiving, though even here thick trunks sprout less easily than fruit-bearing twigs. Research into plants and plant telomeres continues...

Almost enough for this short article. Just an aside about the organelles. The DNA in the nucleus is inherited through plant sex (egg + pollen → seed), but the DNA in the chloroplasts and the mitochondria are carried across from the mother plant (the seed, fruit, capsule or pod parent). What do they do about telomeres? Well, they have kept an old bacterial trick: the organelle DNA is organized into a continuous ring (like a circle) so there are no ends! I decline to describe how the organelles replicate!

This article is intended for a home-TC readership, so I have left out a great deal of caveats and exceptions. They are generally not common, so I ask biological scientists to forgive me. The plants that eat animals, those that move around, the sea grasses, and all the other beautiful plant life of this planet will forgive me, I know. Animals and fungi are just minor pests to deal with.

How to make a Growth Chamber — Transfer Box with Cart (Part 2)

Kent Fenley, IN, USA



Tools List: Table saw, circular saw, jig saw, miter saw, cordless drill with good selection of bits and right angle tool and strait edge measuring tools. Four clamps (I could have used more) were used when gluing pieces together.

The **Supplies List** will vary with the type and size of the HEPA Filter. I used a four by eight foot sheet of high density particle board that was used for the sides and ends of the filter box and a half sheet of thin plywood for the top and bottom of the box. Plywood ½ inch thick for the aquarium front was purchased as a half sheet of plywood. I used a bottle of wood bonding Gorilla glue and five tubes of silicone adhesive along with one small double tube of epoxy to bond wood to glass. Aquarium, cart, blower, aluminum duct with plastic ends for attachment to filter box and aquarium and glass for sliding doors on aquarium and one piece of wood molding for notching to create a door channel for the glass. I also purchased screws, nuts and bolts and a new extension cord to wire blower motor.

I started this project with the purchase from a local pet store of a new aquarium that was not too wide. I then ordered from an online source a HEPA filter that was a foot square. I ordered from another online source a blower that was recommended for moving enough air through the HEPA filter, this blower was larger than I expected it to be. Due to the size of the blower I planned the box that surrounded the HEPA filter to be large to carry the weight of the blower. I then purchased a cart from an online source that was large enough to carry the aquarium so that no part of the aquarium would hang over the cart.





I cut the thin plywood and high density particle board for the sides and ends of the HEPA filter box. I used poster board to create a pattern from the HEPA filter. I then used the blower and plastic duct end connectors to map the placement for each end hole on the end pieces and then cut the holes.



Gluing the filter box together was the next step that started with gluing the HEPA filter to the bottom piece of thin plywood. After that piece dried the sides were glued one piece at a time giving ample time for each side to dry before the next piece was applied. The HEPA filter was large enough to provide good support when gluing with clamps. I did not want to use mechanical fasteners like screws to eliminate saw dust from getting on the HEPA filter until the box glue-up was complete. The drilling and screws were applied on the top and bottom only; the edges were carefully drilled and screwed to insure the saw dust would stay outside the box.





The front to the aquarium was cut from $\frac{1}{2}$ inch thick half-sheet of plywood. I placed the plywood on the aquarium opening and traced around the opening to fit the aquarium. I had two pieces of glass for the doors and I traced around the glass on the plywood, then I traced an opening that was $\frac{1}{2}$ inch smaller on every side for the openings. This insured that the glass would cover the holes cut for the openings in the plywood. The larger opening is where I will be working with the plants during the transfer process and the smaller opening will give me free access to the entire aquarium but will normally remain closed during transfers.



Small blocks of wood were cut then bonded with epoxy to the aquarium glass on the inside of the aquarium. This will anchor the front to the aquarium and looks good because the blocks are on the inside of the aquarium. After the blocks were applied I drilled holes and used screws to screw the front to the aquarium. I used silicone adhesive to bond the plywood to the plastic of the aquarium before I screwed on the front on the aquarium.





Wood molding was cut on its back side to provide a channel for the glass doors to slide in (I used a table saw to accomplish that). This molding was cut into pieces to surround the glass and provide a slot for the glass to rest. The pieces were screwed to the front after applying a bead of silicone adhesive to the underside of the molding.

The aluminum duct and plastic attachments for each end was applied using silicone adhesive generously applied to the pieces and screws and bolts to fasten the plastic ends. A prefilter was cut from an air conditioner filter and sandwiched between the blower and filter box. This will catch large particles like hair and lint from entering the filter box.





The blower was turned on to test the unit after the silicone had dried. The blower was fairly quiet when operating and the volume of air exiting the large door was excellent when the door was fully open. The smaller door air flow was not as good when the door was fully open however this door will be closed when transferring plant material. I will use petroleum jelly to seal the doors when the unit is not in use and I must say that I am very happy with the way this project has turned out.



All pictures © Copyright Kent Fenley.

An idea becomes reality. Why not use it?

Frank Tromble, USA

It's been a long time coming. The idea, posted Sunday Feb 10, 2008 (message #14822 on the HTCG forum) by Dr Ronald de Fossard suggesting a wiki-style database for plant tissue culture has finally become a reality. The Free Plant Tissue Protocol Wiki site has arrived. It is in its initiation stage but we see signs of new shoots for sure! You can support it by adding your cytokinins, auxins and procedures at <http://www.PlantTCCases.org> (capitalization is purely optional).

The format of a wiki style database is well suited for entering protocols for plant tissue culture. Wiki pages are searchable and linkable. Even individual words are linkable, meaning you can link something like 'Woody Plant Media' in a protocol to the Woody Plant Media wiki page and look at the ingredients. Looking for molecular weight of meta-topolin? You will be able to click the name meta-topolin in a protocol and find it. It's not all there yet but will be. Many of these things will come in time and we're working on it! As this site grows the information and related information will make it a very powerful tool for anyone who is involved in plant tissue culture. Need it? Of course you do! Secrecy has no place in plant tissue culture, nor has having things hard to find.

Anyone, registered or not, can view the protocols and the other wiki pages. Once registered to the Free Plant Tissue Culture Protocol site, anyone can post a protocol or post other information relating to plant tissue culture.

One small hurdle is that most people are not familiar with the wiki language. Actually it is rather simple but if you are not familiar with it, it doesn't seem to matter how simple it is. It becomes a barrier, even if a small one. The tags that create the Wiki pages can be confusing if you haven't used them before. So we have created a cut and paste template with the Wiki tags already in place. All you have to do is type in your words. Yes, just plain typing. That's it. Of course we are on hand to answer any questions in case you encounter something unexpected or want to do something differently. Please feel free to email me at fbt@frankandjackie.com as I will be happy to help. If you don't want to add the protocol yourself, I am willing to add it for you giving you credit by placing your name as the contributor to the protocol.

If you want to do it yourself but need the template to help you along, type 'template' (all lower case) in the search field located on the left side of the page. The template page will appear. Click the **Edit** tab at the top of the page. **Select all** of the text and copy it into your own page.

To create your own Wiki page about a plant you have worked with, search the name of the plant. An example would be to search for 'Anthurium'. This will bring you to the Search page saying, "There is no page titled Anthurium. **You can create this page**". Click the red letters "**You can create this page**". When the page opens, paste the text from the template and fill in the blanks. Click the **Save** button at the bottom and you have created your Wiki page.

Let's all make it a personal project to culture the Free Plant Tissue Culture site with 24 hours of 3,000 lux fluorescent light, 25°C and make it grow. We need hundreds and thousands of protocols to bloom...

Fern Propagation

Arthur Sale -- Tasmania, Australia

Strictly speaking, this article is not about tissue culture. But so many people want to propagate ferns, and as it is such a different area of plant culture, this is how you grow a species fern from spores. Since ferns produce millions of spores, true tissue culture with all its risks can then be reserved for propagating a special cultivar.

Ferns have been around longer than the gymnosperms (cycads and conifers) and the angiosperms (flowering plants). Yet their habits have evolved just as much as their newer cousins and they remain very successful plants (more than 10,000 species). The first important thing to know about ferns is that the plant we know as a fern (it is a *sporophyte*) is sexless and produces *spores*, contained in *sori* (singular *sorus*) on the underside of some leaves. When they are ready, the sori open and the spores are blown around by the wind. No flowers, no ovaries, no pollen.



If the spore lands on a wetish spot, it may grow. And here is the second thing you need to know: the spore grows into a second small insignificant plant called a *prothallus*, which develops both a female part and male parts. A prothallus looks like a small patch of green. The prothallus does not look like a fern; indeed it is almost as different as it could be. When the prothallus has grown enough, it releases the sperm on its underside, and they swim around looking for an ovary to fertilize. This is the third thing you need to know: at this point, the prothallus or *gametophyte* generation must have free water, whether from rain, waterfall spray or in a plastic box.

If fertilized, the ovary grows into a normal looking fern, and the prothallus dies. Fern sex involves an alternation of generations: fern → prothallus → fern → prothallus...

Because the germination of a spore and the fertilization of a prothallus is a chancy business (remember the water?), some ferns have evolved a backup plan: a vegetative method of propagation. Hare's foot fern (*Polypodium* sp.) sends out runners that produce suckers. Their roots can also be divided, and lend themselves to TC. The Mother Spleenwort (*Asplenium bulbiferum*) grows little plantlets on some of its frond tips that fall to the ground or lie on it and grow.



Propagating

Now you know almost all you need to propagate a fern. The rest is routine. Buy yourself a flat transparent plastic tray with a lid (a food storer is good), and put 1 cm (½") of vermiculite in it. Sterile is good, but it's not absolutely vital. Clean sand or clean kitty litter would do too. Collect some fern leaves with mature sori and put them into an envelope to dry out and release the spores. Sprinkle the spores evenly over the vermiculite and spray with water until the plastic box is wet. Put the lid on. You now have a box which is damp, has thousands of spores, and has a high humidity. Prothalli should grow. Warm is good, but not too hot. Even cold is ok. Every so often, take the lid off for a short time to exchange the air and spray inside to keep it wet. Keep the box in shaded light (for example under 75% shade cloth).

As a variation on this, you could use chopped up chunks of fertile fern leaves with the sori facing down. Just don't try to grow in sphagnum moss or you'll end up with a big crop of moss.

When the prothalli are beginning to grow, make sure that there is a small amount of free water in the mix. Those sperm need to swim to a neighboring prothallus.

The next thing you will notice is tiny fern-like things growing. Let them grow to at least 1 cm (½”) but remember they have not much in the way of mineral nutrients in this box – just photosynthesis. When they look strong enough, tweezers transfer the bigger ones to a new plastic box with seed raising mix and traces of minerals. Keep the mother box, because more ferns may grow from it and can be transferred in turn. You may end up with hundreds if not thousands. Let the now independent ferns grow until they reach near the top of the box, and then slowly acclimatize them to the world (low humidity is a big shock) by opening the lid ever so slightly. Spray and slap it on again if they wilt. Eventually the ferns will be tough enough to face the world and can be planted into small 50mm (2”) tubes with regular potting mix. Congratulations -- you now have nursery size ferns, and it has taken you about a year or less of nurturing. However, ferns won't mind too much if you go on holiday, provided they are kept wet and shady.



Diseases? Hardly any. Ferns survived the dinosaurs munching on them – they will probably survive you. Competition: it would be surprising if some mosses didn't grow in the tray as well, and you may get a few ferns that you did not expect – they are very opportunistic at finding wet conditions. Size? Some ferns grow into dense low mats like carpets, for example *Blechnum pennamarina*. Others grow into trees, such as *Dicksonia antarctica* and *Cyathea cooperi* (see right). Then there are the stag-ferns which grow as epiphytes, the challenging filmy-ferns (need lots of water), bracken, and so on. Temperature: some like it hot, some like it cold. All like water, which is their trademark.

NOTE. All photographs © Copyright Arthur Sale.
No ferns were harmed in the photography for this article.





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