

# Tissue culture in the Home Kitchen

it's not as hard as you might think!

A slide show by Rick Walker

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## Why bother with Tissue Culture (TC)?

- faster propagation (perhaps 10x) - reduces demand for wild collected plants.
- no worry about fungus, pests, etc.
- long term maintainance of plants (stick 'em in the fridge!)
- IDENTICAL clones of horticultural varieties easily generated

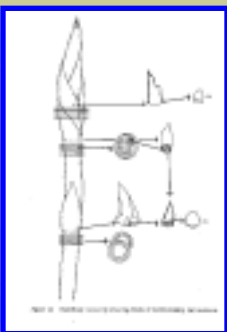
(actually, the last point is not quite true. Scott Hyndman, [marva@nebula.ispace.com](mailto:marva@nebula.ispace.com), informs me that Clonal integrity is influenced by stress factors, genotype, culture age, cutting and transferring technique, and numerous other factors still being investigated in the voluminous scientific literature on just this fascinating aspect of plant tissue culture alone.)

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## Some useful definitions



Totipotency: certain cells have the capacity, when isolated and properly grown, to regenerate a whole plant. This is nothing strange or unusual. The picture shown here shows how the common "spider plant" is capable of starting new growth at the end of each shoot.



Meristem: The region in a growing plant where the cells are rapidly dividing. The picture shows how the *meristem* can be isolated from a strawberry shoot to be grown out in sterile tissue culture.

Both these pictures are reproduced from "Plants from Test Tubes" by Lydian Kyte. This book is highly

recommended as a reference for beginners, and a full bibliographic reference is given later in this talk.

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More accurately, this should be called a talk on "in-vitro" propagation of carnivorous plants. "In-vitro" means "in glass", or under sterile conditions.

It is very difficult (or sometimes nearly impossible as in *Nepenthes*) to properly sterilize meristem tissue from many CP. Some of them have symbiotic fungi living within their cells. This stuff usually breaks out and overruns the culture when meristemming is attempted from non-sterile material.

For this reason, the most reliable way to start a CP cell line is from seeds.

Once you have a sterile culture going, then you can multiply the tissue with hormones, and re-divide ad-nauseum. For some CP, notably *Pinguicula* and *Sarracenia*, it is possible to do meristem techniques with normally grown plant material. I won't cover the dissecting procedure here, but the sterile technique, media preparation, etc., are identical to the in-vitro procedures shown here.

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## More useful definitions

### Auxin

hormone that primarily controls cell elongation inhibits side shoots, produced at apical meristem

### Cytokinin

hormone that primarily stimulates cell division. Examples are 6-(y,y,dimethylally-amino)-purine (2iP), Kinetin (K), and 6-benzylaminopurine (BAP)

Toby Marsden defines the three classical stages of hormone-assisted TC as:

- Stage I. establishment/germination (no hormone)
- Stage II. multiplication (low auxin, high cytokinin)
- Stage III. rooting (high auxin, low cytokinin)

For home tissue culture, hormones are not necessary. Most plantlets such as *Pinguicula* already grow much faster in TC than in soil. Unless you need extremely fast multiplication for commercial purposes, or are experimenting with meristem propagation of very difficult species (eg: *Nepenthes*), it is unlikely that you will need to use hormones or cytokinins. Many of these chemicals are dangerous (mutagenic or carcinogenic), and are not really appropriate to be using in the kitchen.

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[Commercial TC media](#)

This photo shows a package of Murashige and Skoog (MS) Tissue Culture media as sold by SIGMA chemical company. Each packet provides enough chemicals to prepare 1L of media. To use this media for CP, it generally needs to be diluted in strength. I usually use 1/2 strength for most non-critical plants. The four bottles shown each contain 1/4 of the SIGMA packet, [built back up to proper sucrose and agar concentration](#). For most CP, 20-30g/L sucrose and 6g/L agar is appropriate.



[A scale, sugar and agar.](#)

Each bottle then conveniently contains the proper chemicals for a 500mL batch of media.

The next slide covers the functions of the major nutrients in TC media.

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## Components of TC growth medium

The Organics: C, H, O

AGAR - neutral substrate

sucrose - (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>) provides energy source for cells

inositol - (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) simple alcohol sugar

hormones - control growth

fungicides - control contaminants

antibiotics - control contaminants

antioxidants

Inorganic *Macro* nutrients

N ... leaf growth, chlorophyll, amino acids, proteins, cell membranes

P ... meristem growth, fatty cell membranes, DNA production

K ... cell division, root formation

S ... root development

Ca ... pectin (cell wall glue) vital to meristem growth

Mg ... integral to chlorophyll molecules

Fe ... vital to pigment and chlorophyll formation

Inorganic *Micro* nutrients

B ... Sugar movement within plant  
Mo ... Nitrogen Fixation  
Mn ... subtle (controls vital enzyme pathways)  
Cu ... subtle (controls vital enzyme pathways)  
Zn ... subtle (controls vital enzyme pathways)  
Cl Al Na Si Co (secondary: need for these varies by species)

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## Using baby-food jars for a growing chamber



[punching an air hole in the lid](#)



[using foil as a contaminant barrier](#)

Baby-food jars make excellent and inexpensive growing chambers. For the beginner, a hole may be made in the lid with a nail and hammer. To prevent contamination, the lid is kept wrapped with aluminum foil. This allows slight breathing of the media while providing a baffle to exclude bacteria and spores.

The more advanced TC enthusiast may wish to purchase plastic caps from a commercial source such as SIGMA chemical company. I use the "MAGENTA B-cap". They are sterilizable, provide baffles, and are transparent to light. This makes it easy to grow the cultures with overhead illumination. These caps are designed to be used with standard baby-food jars.

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## A Simple Home Recipe:

Tissue culture does not need to be a "high-tech" affair. Many non-fussy plants can be easily grown on a cookbook "kitchen-style" media. Here is a simple one, adapted from Kyte, to try for *Pinguicula* and *Drosera*. There is a lot of room for experimentation, and this formula can certainly be improved with some trial and error.



### components of a simple homemade media

- 1/8 cup table sugar
- 1 cup water
- 1/2 cup stock solution (Miracid diluted 1/4 tsp in 1 Gallon water)
- 1/2 inositol tablet (125mg)
- 1/4 vitamin tablet with thiamin
- 2 Tablespoons agar flakes

For variation, try substituting coconut milk in place of some of the water. Common concentrations used in the literature are 100 to 200 ml of Coconut milk per liter of media.

Inositol is a common human food supplement and can be found at health-food stores in gel-caps. Agar is sometimes available at the same stores in bulk bins. Another place to look for agar is at Oriental food specialty stores. Agar is commonly used in Asian cooking as a gelling agent for desserts. Get the whitest and purest, unflavored variety that you can find.

You may have to experiment with the concentration when you use non-standardized agars. Your goal is to achieve the minimum concentration of agar that still reliably forms a gel. This will produce a media that offers minimal resistance to root growth.

Note: This recipe was originally designed for general purpose use. Most CP require a more dilute nutrient concentration than non-CP, so you might try reducing proportions of stock solution and sugar until best results are obtained.



### Plants from Test Tubes

This recipe is adapted from the highly recommended book "Plants from Test Tubes" by Lydian Kyte, published by Timber Press (see references at the end of this document for ordering information).

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## **A More Sophisticated Recipe:**

This is the *Pinguicula* Media recommended by Bill Carroll in the Carnivorous Plant Newsletter, v11 n4, December, 1982 pp. 93-96. As you can see, you probably don't want to attempt this one without an accurate balance and a well-stocked supply of chemicals.

- CaNO<sub>3</sub> 1000 mg/liter
- NH<sub>4</sub>NO<sub>3</sub> 300 mg/liter
- KH<sub>2</sub>PO<sub>4</sub> 250 mg/liter
- MgSO<sub>4</sub> 250 mg/liter
- MnSO<sub>4</sub> 10 mg/liter
- Fe Chelate 20 mg/liter
- Thiamine 10 mg/liter
- Inositol 100 mg/liter
- Sucrose 20,000 mg/liter
- Agar 12,000 mg/liter

For shoot multiplication use Kinetin or 2iP in range of 0.5 to 2.0 mg/liter. Auxins for rooting were IBA or NAA in range of 0.1 to 1.0 mg/liter.

Bring media to boil while stirring constantly, dispense into test tubes or other containers and steam sterilize for 15 minutes at 15PSI (120C or 250F).

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## Some commercial sources

### *SIGMA Chemical Company*

P.O. Box 14508, St. Louis, MO, 63178, (800) 325-3010 (ask for the "Plant Cell Culture Catalog"). They tend to be a bit uptight at this company, so you may need to get a "fee-exempt" nursery license from your local Agriculture Department before SIGMA will do business with you.

GIBCO/BRL Life Sciences  
(800) 828-6686

Carolina Biological Supply  
Burlington, NC, (919) 584-0381, 1-800-334-5551, or caroscpub@aol.com

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## Mix and Dispense the Media into the Jars

Whatever formula you use, you need to mix it up according to the manufacturers recommendations, or according to your formula. I usually heat the water almost to boiling before adding the Agar with vigorous stirring. When fully dissolved, you can dispense 1-1.5 cm of media into each Baby-food jar. Be careful not to get any media on the rim or sides of the jars as this will later provide a path for contamination. Replace the (vented!) lids on the jars and stack them into the pressure cooker for sterilizing.

## Sterilizing the Jars and Media



[Loading a simple pressure cooker](#)

Make sure and use a trivet to hold your jars up off the bottom of the cooker. You want to steam-sterilize them, not boil them!

This small, skillet-sized pressure cooker came from the Whole Earth Store for about \$200. It's a fairly pricey, high-end stainless-steel model for gourmet cooking. I included it here as an example of "making-do" with what is available. This canner is just big enough to barely fit 7 short baby food jars.

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[Loading a bigger "Home Canner" pressure cooker](#)

This is a bit more industrial sort of canner, made by "American Aluminum Company". It's very nice for sterilizing big batches of media, tools, and for sterilizing rinse water.

I purchased this one mail-order from Mellinger's [see refs. for address], but the same canner is also available locally at Orchard Supply and Ace hardware. Smaller units from the same company can be had for around \$70 or so.

Although Microwaves have been used for sterilization (see bibliography), the results have been spotty. The dividing line between achieving sterilization and flash-overboiling the media is extremely fine. Even under the best circumstances, the incidence of contamination is much higher with a microwave than with a pressure cooker. If your environment has a high concentration of heat-resistant spores, then microwave sterilization will probably be unusable.

With a pressure cooker you can assume, with near certainty, that your media is absolutely sterile. Then you can focus your attention on reducing contamination during seed sowing, cell transfer, etc. I highly recommend using a pressure cooker for any serious TC work.



[Processing](#)



[Processing](#)

Whichever type of pressure cooker you choose, they all should be set for 15 PSI/250F, and run for 15-20 minutes.

I usually leave the check valve open until it starts to discharge a good quantity of steam. At that point, you are assured that the vessel is well-filled with live steam. You can then close the valve, lower the heat, and start the timer once you've come up to pressure.

*Never leave a cooker unattended. Please read all the safety directions for your cooker before starting!*

Make sure and let the cooker come to room temperature before opening the vessel. If you don't, then your media is likely to burst into a boil and foam all over the place. I usually leave the cooker overnight before opening it. This also has the advantage that the agar will be fully gelled, and there will be no problem with spilling the media when removing the jars.

After things have completely cooled, you can safely open the cooker. There may be a slight internal vacuum which could suck in contaminated room air. It is suggested that the cooker checkvalve be wrapped in paper toweling that has been soaked with isopropyl alcohol. Carefully release the internal vacuum by opening the check valve. The room air will then be filtered by the paper toweling.

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## **Sterilizing Seeds or Tissue**

- Common Isopropyl "rubbing" Alcohol - full strength
- Chlorine ("Clorox" brand bleach dilute 10:1)
- H<sub>2</sub>O<sub>2</sub> (3% Hydrogen Peroxide from the drug store)
- Wetting agent (such as Kodak "photoflow", or liquid detergent such as "Joy" brand)





alcohol, bleach & peroxide



labelled seeds on filter paper



folding paper and securing with plastic clip

I sterilize seeds in a little folded packet of filter paper by soaking for 5 minutes in isopropyl alcohol (frequently shaken or stirred), 2-4 minutes of 1/10 Clorox solution, and 1-2 minutes of 3% H<sub>2</sub>O<sub>2</sub> as a final rinse. I leave the residual peroxide on the seed as a further infection prevention measure. Some workers prefer to rinse all traces of chemicals off the seeds with pre-sterilized water.

It can help to add a drop of detergent to the bleach solution to allow better wetting of the oily seed coat.

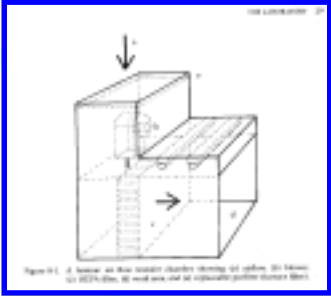
In private correspondence, Jan Schlauer has recommended judging the bleach timing by looking at the color of the seed coat. When you have just noticed a change in color (from black to brown, or from brown to straw-colored), this is about the right time to stop the chlorine disinfecting step.



soaking seeds

It is always a delicate juggling of trying to kill the contaminants without killing the seed. For the best chance of success, you may want to divide your seed into several batches. Process one batch for 1 minute, the next for 2, and the last for 4 minutes. Sow them in separate flasks and keep good records. This will help you to perfect your judgment and technique.

## Doing the actual inoculation



[A commercial laminar-flow hood](#)

This is another line drawing from Lydian Kyte's book: "Plants from Test Tubes". A commercial hood like this can cost perhaps \$1500.00. You can buy the filter and fan units separately to make your own for much cheaper if you are handy with working in plexiglass.

In a laminar-flow hood, the incoming air is filtered by a High-Efficiency-Particulate Air Filter (HEPA) and flows smoothly over the work area. The HEPA filter is fine enough to completely remove mold spores and bacteria from the air stream.

John Laroche has written a simple "howto" describing a how to build a [Glove box, Laminar Flow hood and a culture rotator](#).

For the hobbyist, good results can be obtained with the much simpler system illustrated below. This is an adaptation of the "glove box" type of transfer chamber.



[Simple aquarium transfer box](#)

This is a 40 Gallon Aquarium, turned on its side, and covered with a curtain of plastic sheeting. I use the overhead fluorescent fixture for lighting. The clear section of glass in front of the light fixture is where to look for a clear view.

Prior to using the chamber, you should swab down the inside of the box with a paper towel moistened with Isopropyl. Be *very careful* to let the fumes dissipate prior to lighting your lamp! In this regard, Isopropyl (rubbing alcohol) is much safer than, say, Lysol Spray, which contains Ethanol (grain alcohol) and is much more flammable.

To further safeguard against contamination, you may want to buy a home air filtering unit. The Holmes company makes a unit with a true HEPA filter, for about \$70.00. I usually leave the unit running for a day or so prior to doing sterile transfer work. This greatly reduces much of the air-borne dust in the home environment.

This same chamber serves nicely as a growing area.



[cleaning hands prior to beginning work](#)

I clean my hands with soap and water, and rub them down with isopropyl. Plastic surgical gloves can also be worn, if desired. A short sleeve shirt is recommended to avoid carrying particles in with the fabric.



[tools used for transfer work](#)

A good watch is useful for timing the sterilization steps. (Make sure you think through your technique... I've sometimes gotten involved in sowing seeds, and left others to soak for 20 minutes in bleach... this is not recommended!)

Another useful tool is a pair of tweezers. These should be fairly long so that you can manipulate the material without getting your hands too close to the agar. An 8" piece of thin, stiff wire with tip fashioned into a 1/8" loop is helpful for sowing seeds.

A pair of forceps and a razor blade or sharp knife for dividing clumps of plants may also be needed.



[alcohol lamp for sterilizing tools](#)

A camping burner or at least a candle will be needed in order to sterilize the wire loop during the sowing procedure. I use a lab-style alcohol lamp. This type of lamp has a broad base to prevent tipping. Be careful!

Also, note that Isopropyl alcohol does not burn well at all. You will need to buy denatured methyl

alcohol for your lamp. This fuel burns cleanly and leaves no residue on the tools.



[sterilizing inoculating loop in flame](#)

I usually dip my tools in isopropyl and then "flame" them off in the alcohol lamp. This helps to sterilize both the shaft and tip of the tool. After "flaming" off the isopropyl, I then heat the tip of the loop until it glow red. It can then be inserted in the gel while still hot to cool it down. This technique serves two purposes: 1) it keeps the tip hot to protect from contaminants, and 2) it picks up a little gel on the tip to make it "sticky". This bit of stickiness will help to pick up the seeds in the next step.



[picking up the seeds](#)



[sowing the seeds](#)

Notice the MAGENTA B-CAP held in the curl of the right hand. This is a helpful sterile lab technique that is worth practicing. Here is the procedure:

1. The left hand picks up the capped, sterilized jar with media
2. The right hand dips the tool in a cylinder of isopropyl
3. The little finger and fleshy part of the palm of the right hand is used to open the plastic cap of the culture jar. The lid is NOT set down - this might lead to contamination.
4. It is best if the open jar is held at an angle away from the technician, so that air-borne spores are less likely to be able to settle onto the media.
5. Holding both the lid and the tool, the right hand then flames the tool to sterilize it.
6. The red-hot tool is then inserted into the agar to cool it and make it slightly sticky.
7. The sticky loop is then used to pick up a few seeds from the filter paper and to deposit them

evenly across the agar surface.

8. The right hand, still holding the plastic lid, now replaces it back on the jar.



[showing seed placement](#)

As usual, there is a trade-off to be made here. The more seeds you sow, the better your chance of having a successful germination - BUT - also the higher chance of contamination. For easily sterilized glossy seeds such as *Dionaea*, I usually sow up to 20 seeds. For tougher seeds, like *Nepenthes*, you might be better advised to only sow 3 or 4.

## Transferring plantlets for further growth



[\*Pinguicula heterophylla\* in culture](#)



[tranferring plantlets](#)



[plants in-vitro and also planted out](#)

I have kept my cultures under coolish home temperatures (60-75 degrees F), 12 inches below a two-tube 40W fluorescent light fixture. I keep the culture jars inside the same glass aquarium that I used as the sowing chamber. This helps to reduce contamination from air-borne dust.

After your plantlets have reached the size of a pea, you have a choice of further multiplying them in-

vitro, or transferring them out to grow in regular soil. If you choose to multiply your plants further, you initiate the process by simply cutting up your sterile material and moving it into new media. At each stage, you may be able to increase the number of flasks by over 10 fold.

Of course, all dissection work must be done under sterile conditions. A laminar-flow hood is really handy here, as the plant material will be exposed to possible contaminants for an extended period of time.

If you are skillfull and quick, it can still be done with minimal equipment.

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## Moving the plantlets to regular soil

The key to sucessfully transferring your in-vitro plantlets into soil is to be *very fastidious* about washing off *all* the TC media from the roots.

I usually put the plantlet under running, tepid water, and use the force of the water to thoroughly dissolve off all the old media. If this is not done, then molds will inevitably take hold and overpower your plants.

After planting out, treat the plants the same as they were treated while still in-vitro. A humidity tent made with a zip-lock bag will help the plants acclimatize. Let them stay sealed for a week or so. You can then gradually open up the bag over the course of another week to get the plants used to lower humidity. Once they are "hardened off" properly, you can treat them as any other soil-grown plant.

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## Summary of CP Tissue Culture Formulas and References:

Please excuse the technical format of this list. It is organized by family and genus. Much of the info here is due to Jan Schlauer, Andreas Wistuba, John Laroche and others on the CP listserv group. Many thanks to these intrepid experimentors!

In cases where there is no formula listed, you might try using one for a related genus in the same family. Other than that, you are probably exploring new territory. Please keep good records and let us know what you find out!

Sarraceniaceae {DUMORT.}

- **Darlingtonia** {TORR.}
- **Heliamphora** {BENTH.}
  - 2/3 Knudsen C with 0.1mg/l NAA (reported by Toby Marsden)
- **Sarracenia** {L.}
  - 2/3 Knudsen C(\*) with BAP, ABA for mult and root
  - 1/6 MS, is second choice

Byblidaceae {DOMIN}

○ **Byblis {SALISB.}**

- Sigmas modified MS (1/2 x macro-, 1 x micro-)(M0153) + BAP, IBA sometimes vitrification trouble with *B.gigantea*
- Bunn 1985. Australian Horticulture. 83(5):103

Cephalotaceae {DUMORT.}

○ **Cephalotus {LABILL.}**

- Sigmas modified MS (1/2 x macro-, 1 x micro-)(M0153) + BAP, IBA
- In vitro propagation of *Cephalotus follicularis* (Australian Pitcher Plant). HortScience 14, 521-513

Droseraceae {R.A.SALISB.}

○ **Aldrovanda {L.}**

○ **Dionaea {SOLAND. ex ELLIS}**

- Hutchinson 1984. Scientia Horticulturae 22:189-194.
- Beebe 1980. Bot. Gaz.141(4):396-400.
- Parlman et al. 1982. J.Amer.Soc.Hort.Sci. 107(2):305-310.
- Parlman et al. 1982. J.Amer.Soc.Hort.Sci. 107(2):310-316.
- GERMINATION: 1/2 strength MS Salts, full strength minimal organics, 100mg/l Casien, 100mg/l inositol, 30000 mg/l sucrose and 7 g/l agar Ph at 5.9 Replate medium as above but with 0.2 mg/l NAA and 5.0 mg/l 2iP. - John Laroche

○ **Drosera {L.}**

- 2/3 Knudsen C (\*)
- Janssens 1986. Med.Fac.Landbouww.Rijksuniv.Gent. 51(1):61-66.
- Anthony, J. (1992). In vitro propagation of *Drosera* spp. HortScience 27, 850.
- Jeff Welch reports good results with *D.petiolaris*-complex using 1/4 MS basal salts plus vitamins, 20g sucrose and 6g/l agar.

○ **Drosophyllum {(L.) LINK}**

Nepenthaceae {DUMORT.}

○ **Nepenthes {L.}**

- 2/3 Knudsen C(\*)with 0.2-2mg/L BAP for mult. Rooting horm not neces.
- Anderson's may be used also (*N. ehippiata* likes it).
- Highland ?: 1/3 MS
- Lowland only: 1/2 MS, 20g/L Sucrose, 6g/L agar. w/ 0.1-0.2 BAP multiplying w/ 2mg/L IBA rooting
- Toby Marsden recommends addition of 0.1mg/l NAA to *N. media*

Dioncophyllaceae {(ENGL. & GILG) AIRY-SHAW}

○ **Dioncophyllum {BAILL.}**

○ **Habropetalum {AIRY SHAW}**

○ **Triphyphyllum {AIRY SHAW}**

Lentibulariaceae {L.RICH.}

- Bill Carroll's media (ICPN v11 n4 12/82 pp. 93-96)
- 1:5 MS

○ **Genlisea {ST.HIL.}**

- 1/5 MS for *G.pygmaea*

○ **Pinguicula {L.}**

- Adams et al. 1979. HortScience 14(6):701-702.
- 1/5 MS, 30 g/l sucrose, pH 5,8, Agar

○ **Utricularia {L.}**

- Pringsheim & Pringsheim esp. for aquatics Amer.J.Bot.49:898-901 (1962)
- Carrol's ONLY for large-leaved. sp: U.alpina, longifolia, calycifida

\*= You may add 37,26mg/l Na<sub>2</sub>EDTA and 27,8 mg/l FeSO<sub>4</sub> x 7H<sub>2</sub>O. Andreas Wistuba recommends adding the MS-vitamins to Knudsen C medium.

Note: most media should be prepared with agar at 6g/L, and sucrose at 20g/L.

IBA is an abbreviation for indolebutyric acid

NAA is an abbreviation for naphthylacetic acid

IAA is an abbreviation for indoleacetic acid

MS is an abbreviation for Murashige and Skoog formula. These are all growth regulators for controlling rooting, multiplication, callus formation, etc.

Some of these sorts of chemicals are potent carcinogens and should be treated with utmost respect. I find that for casual home TC, that most of these are not really needed. They are used for really speeding up growth or for getting phenomenal multiplication.

If you wish to try these out, then I recommend really delving into Lydian Kyte's book before beginning.

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## General References:

1. Achieving the sterile state for home tissue culture, Part I, Brian Johnson, CPS Journal 14, 18-19.
2. Achieving the sterile state for home tissue culture, Part II, Brian Johnson, CPS Journal 16, 9-10.
3. Tissue culture of carnivorous plants at Oxford. Steve Woodward, et al. CPS Journal 15, 16-19.
4. Tissue culture of carnivorous plants. Gareth Davies et al. CPS Journal 12, 17-20.
5. In Vitro propagation of the Butterwort *Pinguicula moranensis* Richard Adams et al. HortScience 14(6), 701-702.
6. "In Vitro Propagation of *D. natalensis*". S. Afr. J. Bot. 54(1):94-96 1988. Authors: Crouch, I.J. and Van Staden.
7. Tisserat et al. (1992). Microwave sterilization of plant tissue culture media. HortScience 27, 358-361.
8. R. L. M. Pierik: In Vitro Culture Of Higher Plants KLUWER ACADEMIC PUBLISHERS, P.O. Box 358, Accord Station, Hingham, MA 02018-0358 (ISBN 90-247-3531-9), paperback
9. Hutchinson 1984. Scientia Horticulturae 22:189-194 (*Dionaea*)
10. Beebe 1980. Bot. Gaz.141(4):396-400 (*Dionaea*)
11. Parliman et al. 1982. J.Amer.Soc.Hort.Sci. 107(2):305-310 (*Dionaea*)
12. Parliman et al. 1982. J.Amer.Soc.Hort.Sci. 107(2):310-316 (*Dionaea*)
13. Janssens 1986. Med.Fac.Landbouww.Rijksuniv.Gent. 51(1):61-66 (*Drosera*)
14. Bunn 1985. Australian Horticulture. 83(5):103 (*Byblis*)
15. Rathore et al. 1991. J.PlantPhysiol. 139:246-248 (*Nepenthes*)
16. Adams et al. 1979. HortScience 14(6):701-702. (*Pinguicula*)
17. R.A. Dixon: Plant Cell Culture - A Practical Approach IRL Press Inc., P.O. Box Q, McLean, VA



22101-0850 ISBN 0-947946-22-5 paperback

18. Methods in Plant Tissue Culture by Paul J. Bottino, 1981, 72 pages.
19. Experiments in Plant Tissue Culture by John H Dodds and Lorin W. Roberts, 1993 (2nd ed.), 232 pages.
20. Introduction to In Vitro Propagation by Donald Wetherell, 1982, 87 pages.
21. Carolina Biological Supply has 3 books on plant TC. Their \_800\_ number is 1-800-334-5551
22. "Plants from Test Tubes - Third Edition" by Lydian Kyte and John Kleyn, (ISBN 0-88192-361-3) published by [Timber Press](#), 133 S.W. Second Avenue, Suite 450, Portland Oregon, 97204-3527, U.S.A. (503) 227-2878, (800) 327-5680 (ordering hours M-F 8am-5pm, Sat 8am-noon, Pacific time), fax (503) 227-3070 e-mail orders to: [orders@timber-press.com](mailto:orders@timber-press.com). - Price is US: US\$ 29.95, Canada: C\$ 41.95, Europe: UK 22.50, Elsewhere: US\$ 29.95.
23. Mellinger's Inc., 2310 W. South Range Rd., North Lima, Ohio 44452-9731, (216)549-9861, Order line: 1-800-321-7444. Mellinger's is a general greenhouse/garden supply firm. They offer the large pressure canner illustrated here as a mail-order item.

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[Rick Walker](#)

Agilent Labs, Palo Alto  
[rick\\_walker](mailto:rick_walker) "AT" [omnisterra.com](http://omnisterra.com)

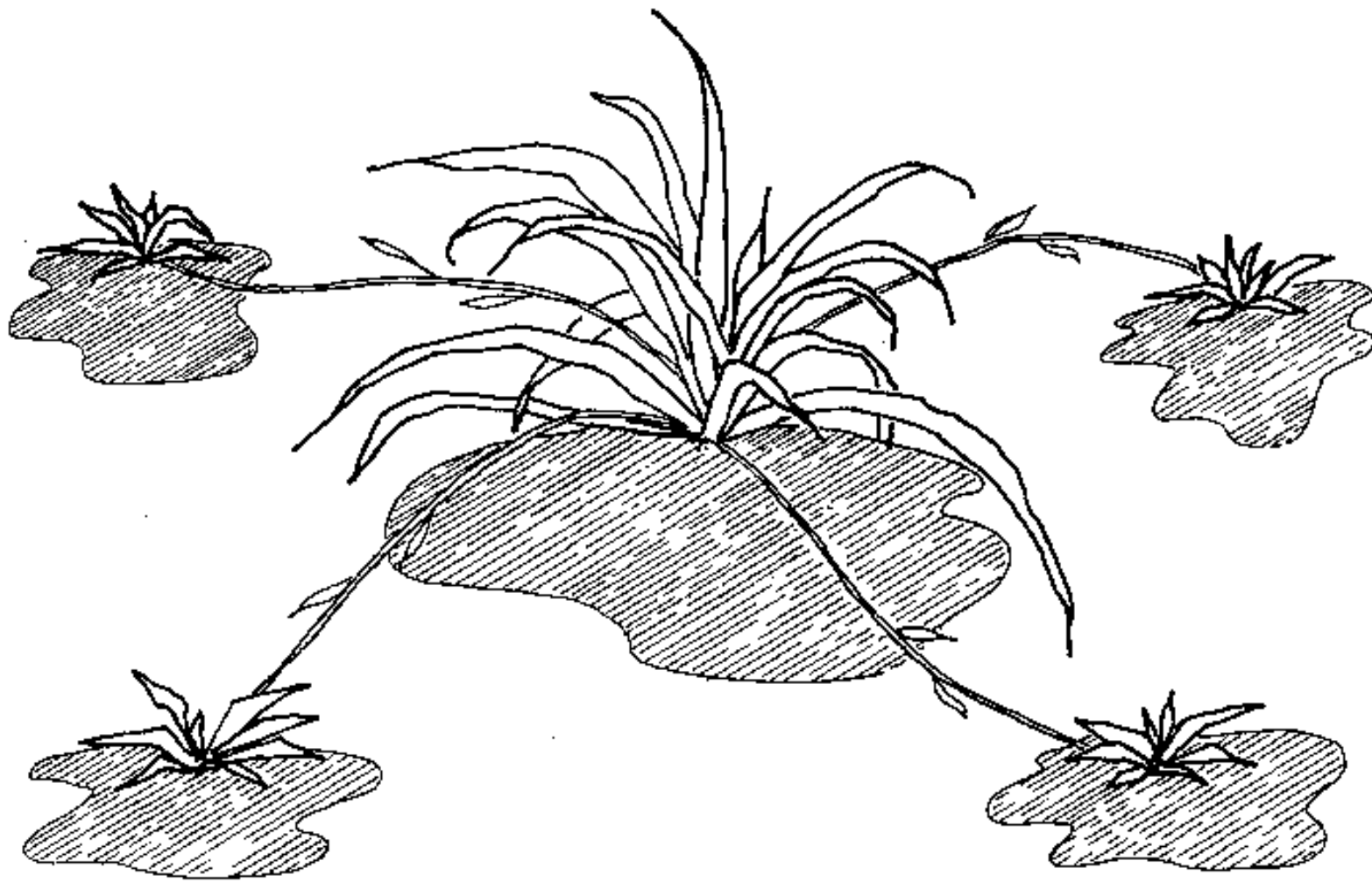


Figure 1. A natural clone. Nature has been "cloning" for eons. Whenever a plant reproduces itself vegetatively it produces a clone.

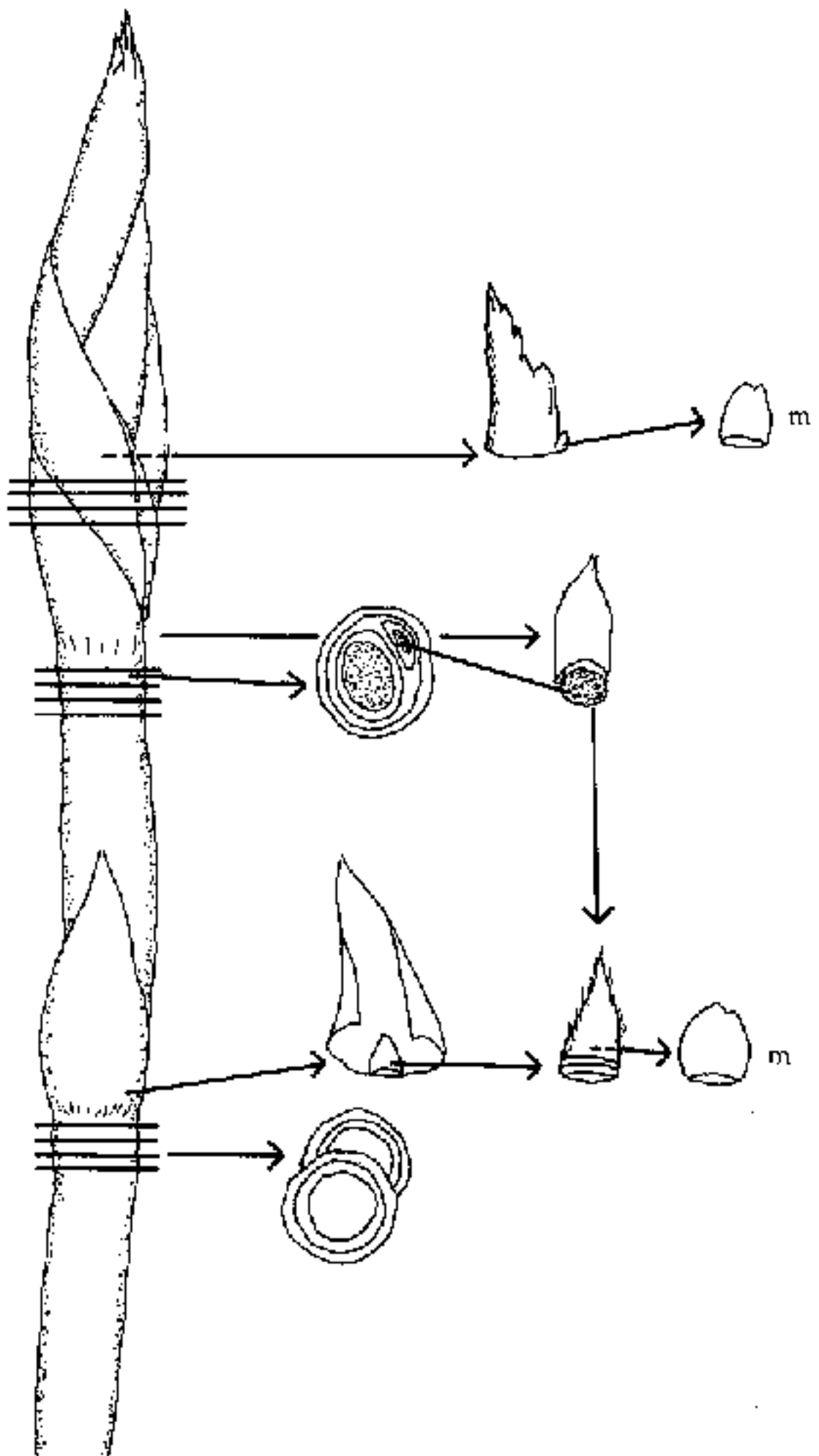
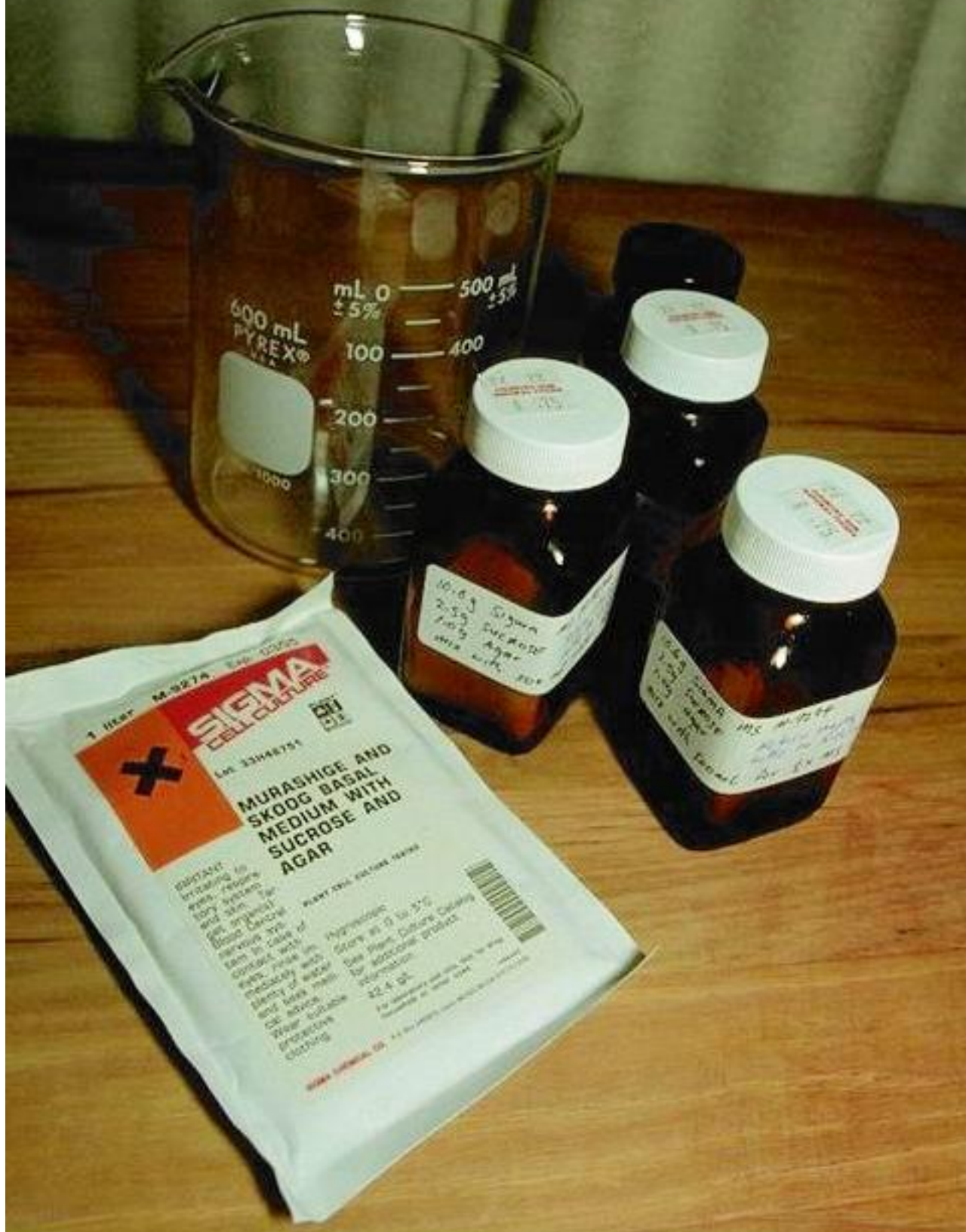


Figure 12. Strawberry runner tip showing details of meristemming. (m) meristem.





600 mL  
PYREX®

mL 0  
± 5%  
500 mL  
± 5%  
100  
200  
300  
400



1 HBSP M-927A Rev. 12/95  
**SIGMA**  
CELL CULTURE

Lot 33448751  
**MURASHIGE AND SKOOG BASAL MEDIUM WITH SUCROSE AND AGAR**

**PRECAUTION:**  
Irritating to eyes, nose, skin, and clothing. Avoid contact with eyes, nose, and skin. Wash thoroughly with soap and water. Wear protective clothing.

**PLANT CELL CULTURE MEDIA**

Hygromycin  
Store at 15 to 25°C  
See Page Culture Media  
for additional product  
information.  
22.4 g



Sigma Chemical Co. 1-800-421-8710  
www.sigmaaldrich.com

10.63 Sigma  
2.53 Sucrose  
1.03 Agar  
with 200

10.63 Sigma  
2.53 Sucrose  
1.03 Agar  
with 200

# How to mix up diluted Murashige and Skoog (MS) media

## starting with premade formula with sucrose and agar

---

Let's say you want to make 1/2 MS formula.

If you buy just MS salts *without* organics, then you simply cut it in half and add enough sucrose to make 20-30g/L in the final concentration, and agar for 6g/L.

If you are using MS packets that *already* contain sugar and agar, then the calculation must take into account how much sugar and agar is already in the packet. You only add back in the balance needed to get to the desired final concentration.

Lets say I have a packet of MS *with* sugar and agar, with an assay of (say) 10 g/L sucrose and 8g/L agar (I just made these numbers up - check with your particular media for your own numbers).

If I cut this packet in half, and add each half to a liter of water, then I'll have exactly the 1/2 MS salts that I want, but the sugar/agar will be weak. In fact, I'll have (per liter):

1/2 MS (just what I want)  
5g/L sugar (1/2 the rated value)  
4g/L agar (1/2 the rated value)

But I really need:

20g/L sugar  
6g/L agar

So I would mix in  $(20-5) = 15\text{g}$  \*extra\* sugar and  $(6-4) 2\text{g}$  \*extra\* agar to each of my two batches.

Actually, I find it convenient to break my packets up into 500ml quantities.

In this case, I'd break up the initial mix into four equal parts and put the portions in dry, brown glass bottles. To each bottle I would add 7.5g sugar and 1g agar. Store in the freezer compartment, and reconstitute with 500ml boiling water.

Nice and convenient.













PURE CANE  
GRANULATED  
SUGAR  
from Hawaii

AGAR  
FLAKES

Sea  
Vegetable  
Gelatin

INOSITOL  
CAPS

Theramins-A  
High Purity  
Multivitamin  
Minerals

PETERS  
PROFESSIONAL

SOLUBLE PLANT FOOD

30-10-10















Al. baumannii

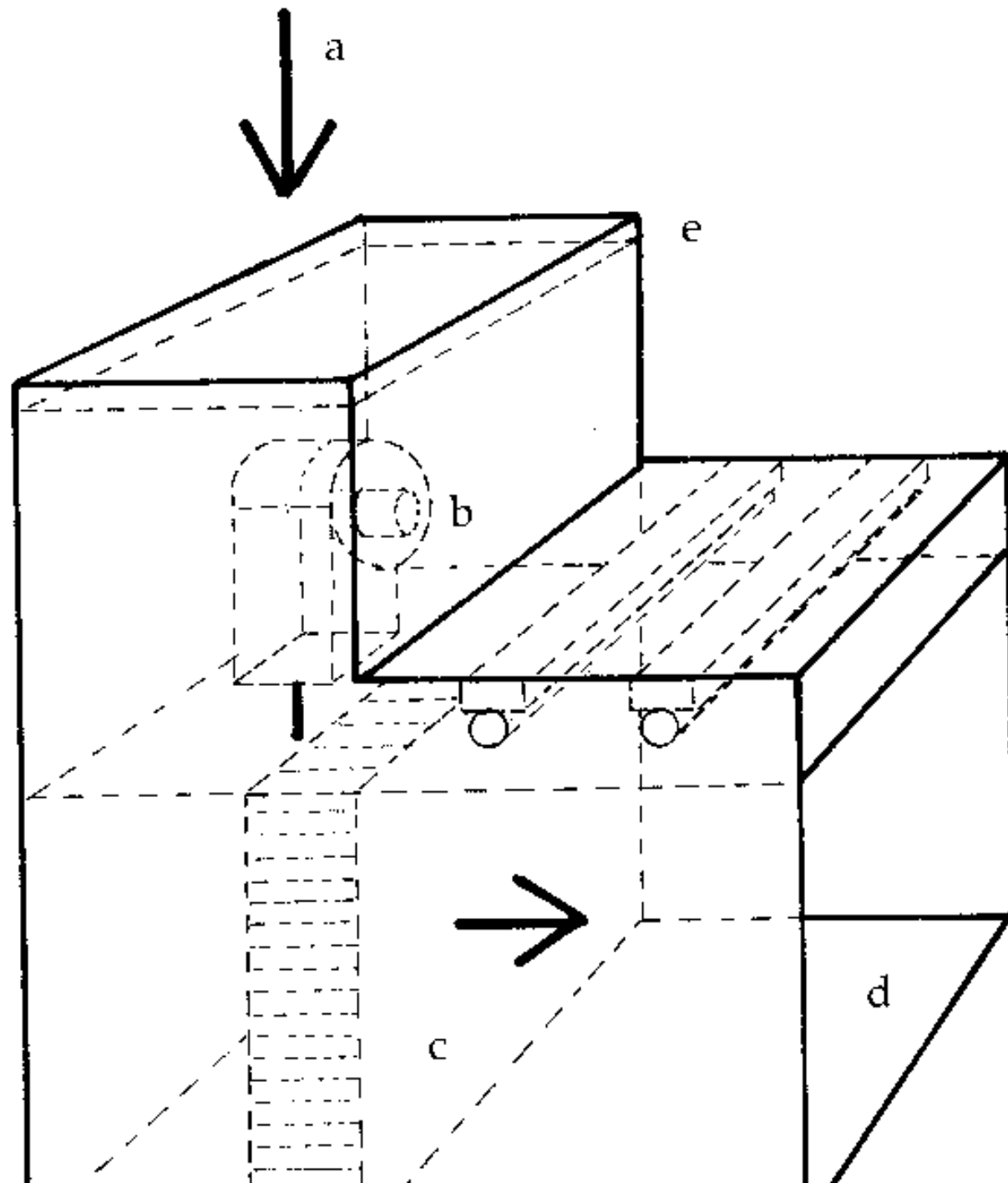
Escherichia coli

Staphylococcus aureus

W.C.  
and another organism







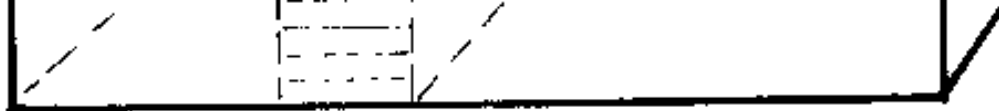


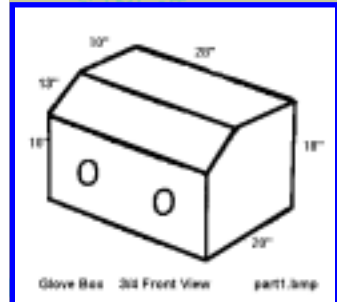
Figure 8-1. A laminar air flow transfer chamber showing (a) airflow, (b) blower, (c) HEPA filter, (d) work area, and (e) replaceable prefilter (furnace filter).

# PROJECTS: GLOVE BOX: LAMINAR-FLOW BOX: ROTATOR

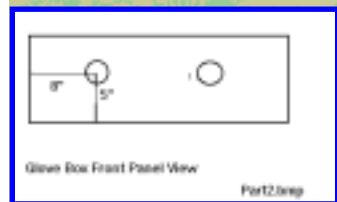
By John Laroche, February 1st, 1995

If you have any construction knowledge, or capability, the diagrams provided on the following pages and the brief commentary below is enough to get you going. Otherwise, give this material to a good handy person and he she can build it for you.

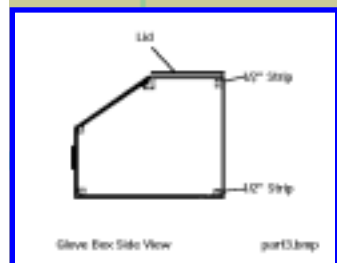
## GLOVE BOX



[Glove box 3/4 front view](#)



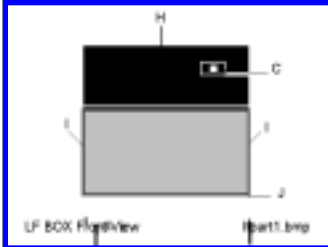
[Glove box front panel view](#)



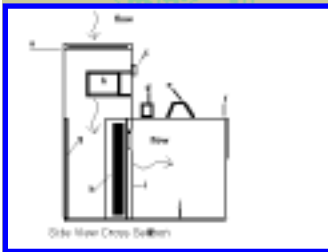
[Glove box side view](#)

This is constructed from clear acrylic; 3/8" thick for the bottom and sides, 1/2" thick for the lid. The plastic is joined by Acrylic Weld a clear solvent that can be purchased where you purchase the plastic. The glove holes should be made to fit the type of gloves you intend to use (there are several kinds available). The small speckled blocks represent 1/2 x 1/2 strips of acrylic which act to reinforce the joints.

## THE CLEAN AIR STATION (LAMINAR-FLOW BOX)



Laminar Flow box front view



Laminar Flow box side view cross section



Conceptual Clean Air Station Design

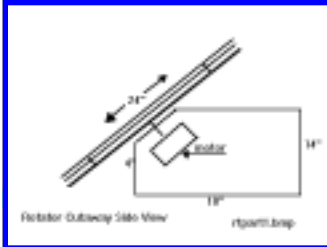
This is a lot easier to build than it appears. Though only a simple diagram and material list it is self-limiting. If it looks too complicated, or you do not understand it; you do not have the skill to build it.

## THE PARTS

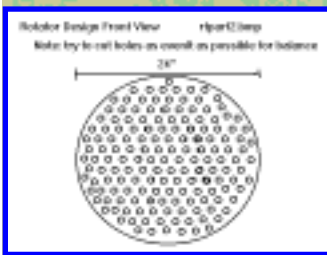
- A Removable Pre-Filter; something like a furnace filter.
- B 300 cfm blower and motor.
- C Control Box; switches for blower, light and UV-light.
- D UV-light (optional)
- E Fluorescent work light
- F Breath Guard; on a hinge; made of 3/8" acrylic.
- H HEPA Filter.
- I 3/8" Clear acrylic sides and top.
- J Bottom work surface; 3/4" plywood covered with high impact laminate.
- K Protective grille for HEPA filter; made of "Egg Crate" Drop ceiling panel.
- L Cabinet.

This is really easy to build if you study a few working models and have an understanding of it's use and of each parts purpose.

## ROTATOR



[Rotator cut away side view](#)



[Rotator design front view](#)

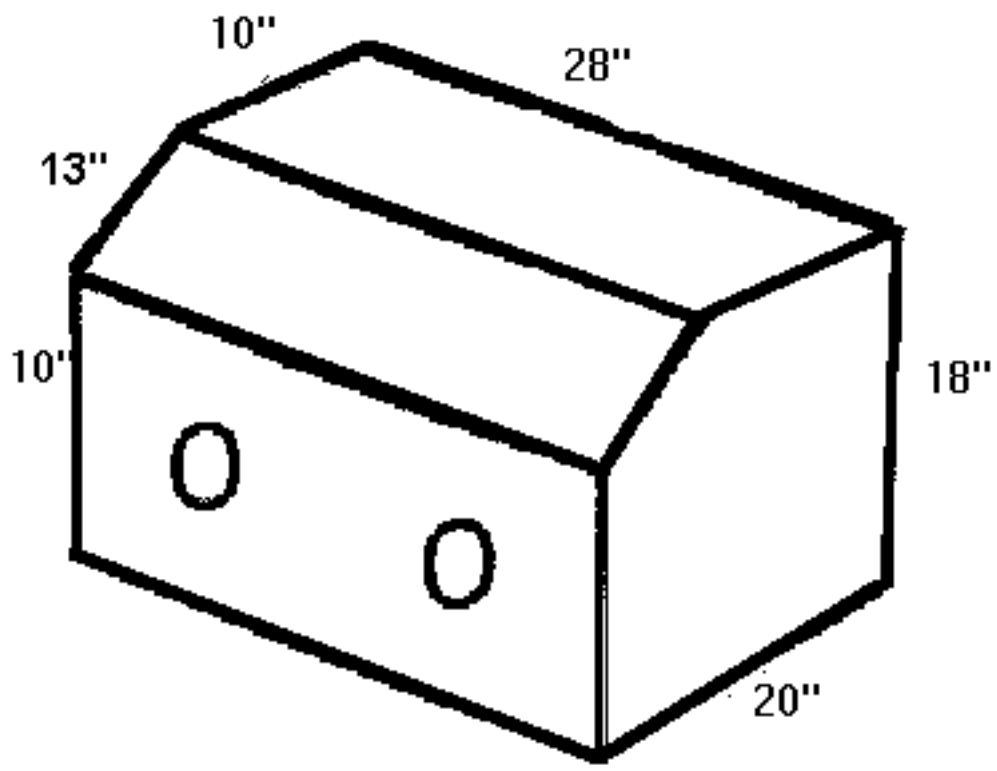
This is a really simple project. The three main parts are: The cabinet/base, the motor and the wheel. The base is a simple plywood box; vented to keep the motor cool. The motor is a gearmotor, which turns at 1 rpm, and is rated for continuous duty. Dayton model 22098 is a fine choice. The wheel is made of three layers. The bottom most layer is 3/4" plywood, covered with laminate, the other two are 3/8" acrylic. It is attached directly to the spindle of the motor by means of a cast sheave. The three layers are separated by threaded rods and nuts (the rod can be covered by an aluminum gutter nail sleeve). The holes for the tubes are made thusly: cover the face side of the plywood with laminate and sandwich all three layers together. Drill clean through the three pieces with a hole saw (27 mm for 25 mm tubes)- Then cover the back of the plywood with laminate and attach the three parts together with the threaded rods. The tubes will sit perfectly on the wheel.

---

[Rick Walker](#)

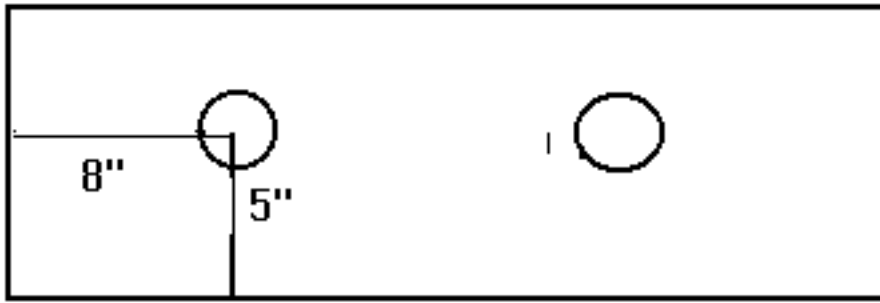
rick\_walker "AT" omnisterra.com





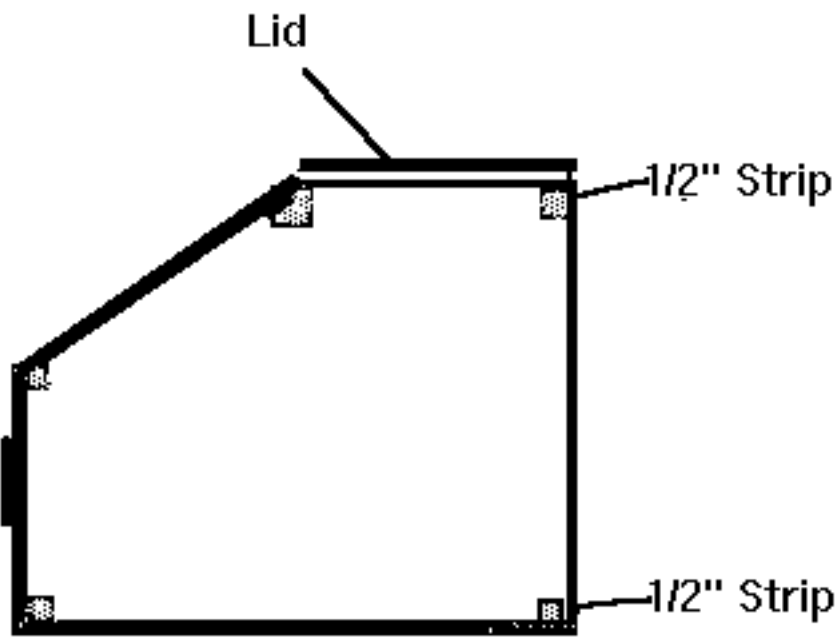
Glove Box 3/4 Front View

part1.bmp



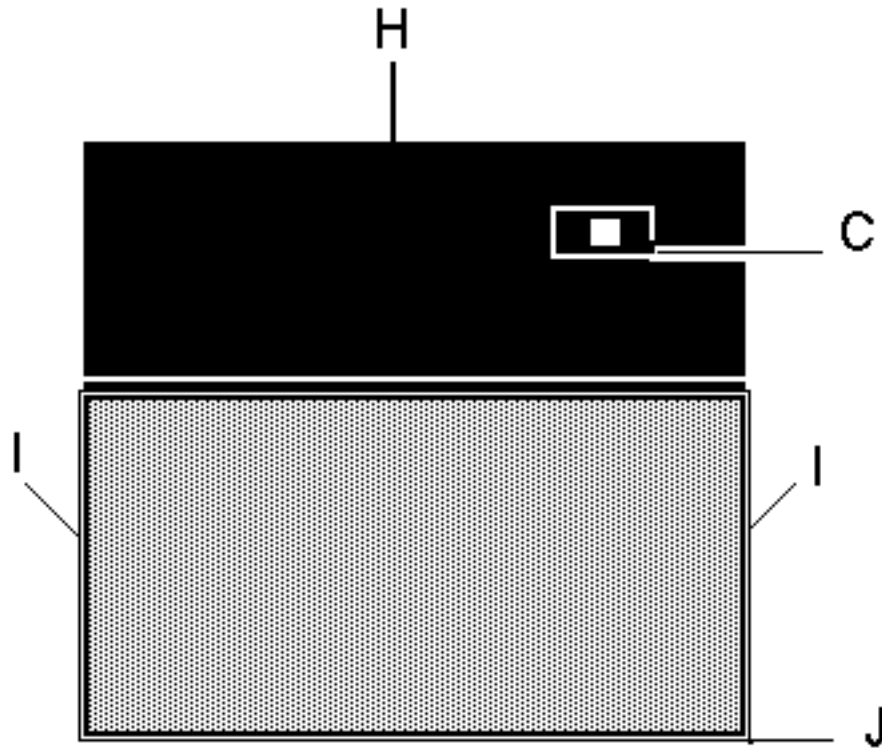
Glove Box Front Panel View

Part2.bmp



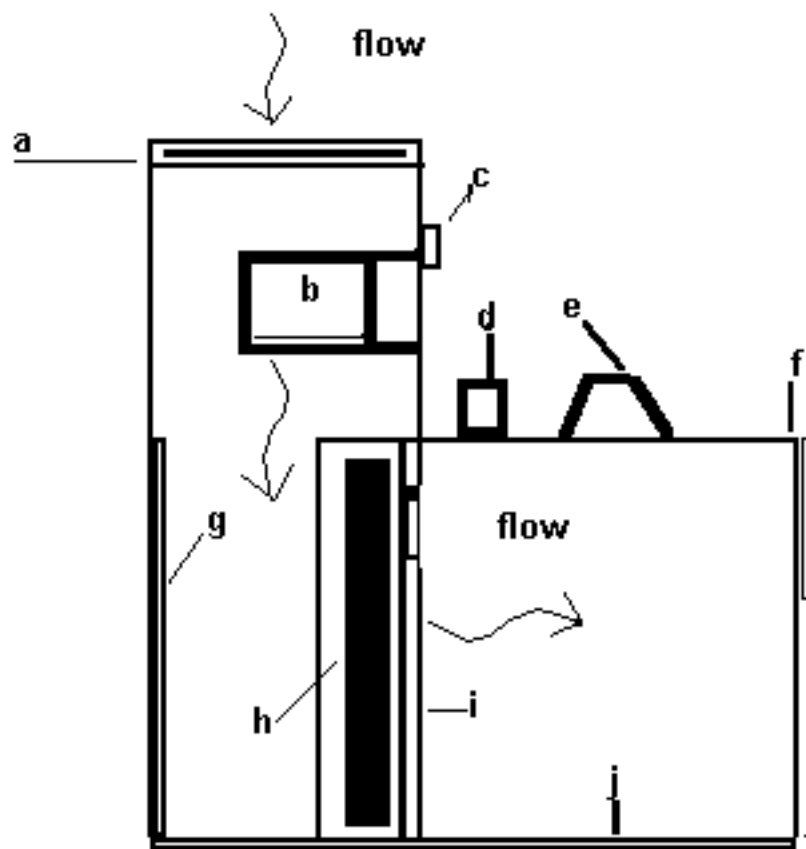
Glove Box Side View

part3.bmp

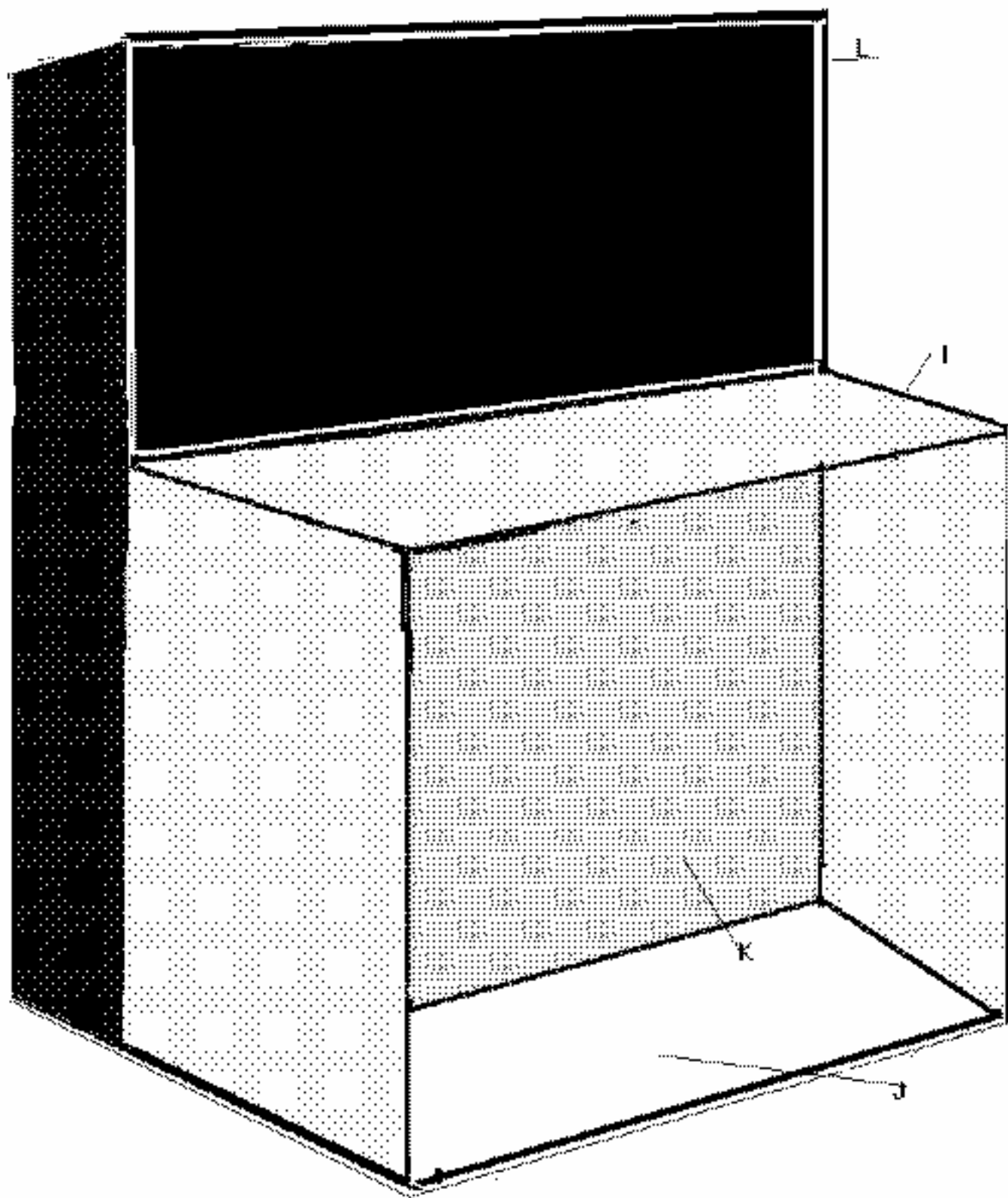


LF BOX Front View

part1.bmp

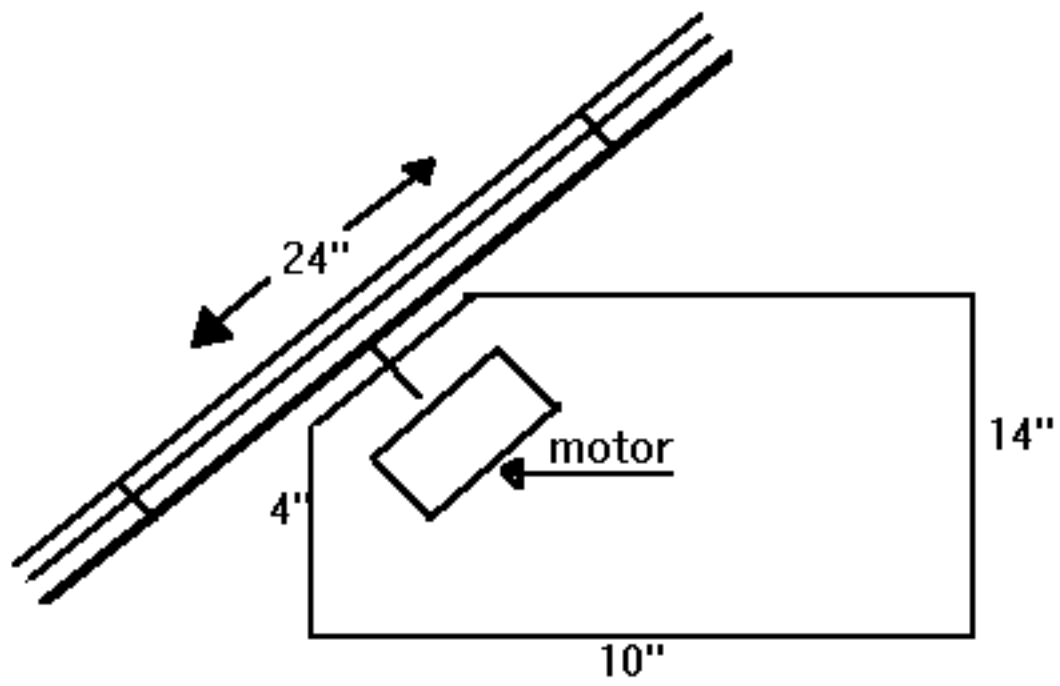


Side View Cross Section



Conceptual Clean Air Station Design





Rotator Cutaway Side View

rtpart1.bmp



Rotator Design Front View

rtpart2.bmp

Note: try to cut holes as eventl as possible for balance

