Iplate Suite Inventory & Draft Protocols

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1 Overview

The following report lists the current inventory and draft protocols for the operation of the "Iplate Suite", which is an affordable and customizable set of devices that employ the "Ichip" design for isolation and cultivation of microorganisms, namely those of soils, using diffusion microchamber arrays. These devices enable the contact of soil or other environmental medium with cells while allowing transfer of growth factors (or "growth crypto-factors" or "the soil crypto-cocktail" as their *in situ* density and interactions in soil solution are poorly known), but the microbes remain isolated for subsequent analysis or transfer. It is the intent of this project to improve the cultivability of soil microorganisms and other "bugs" for microbiological investigations as well as applications to microbial physiology, community ecology, biogeochemistry, antibiotic discovery, and unknown further applications.

There are currently four essential types of Iplate devices available, whose numbering follows their development beginning at the original Ichip (Type 0) and

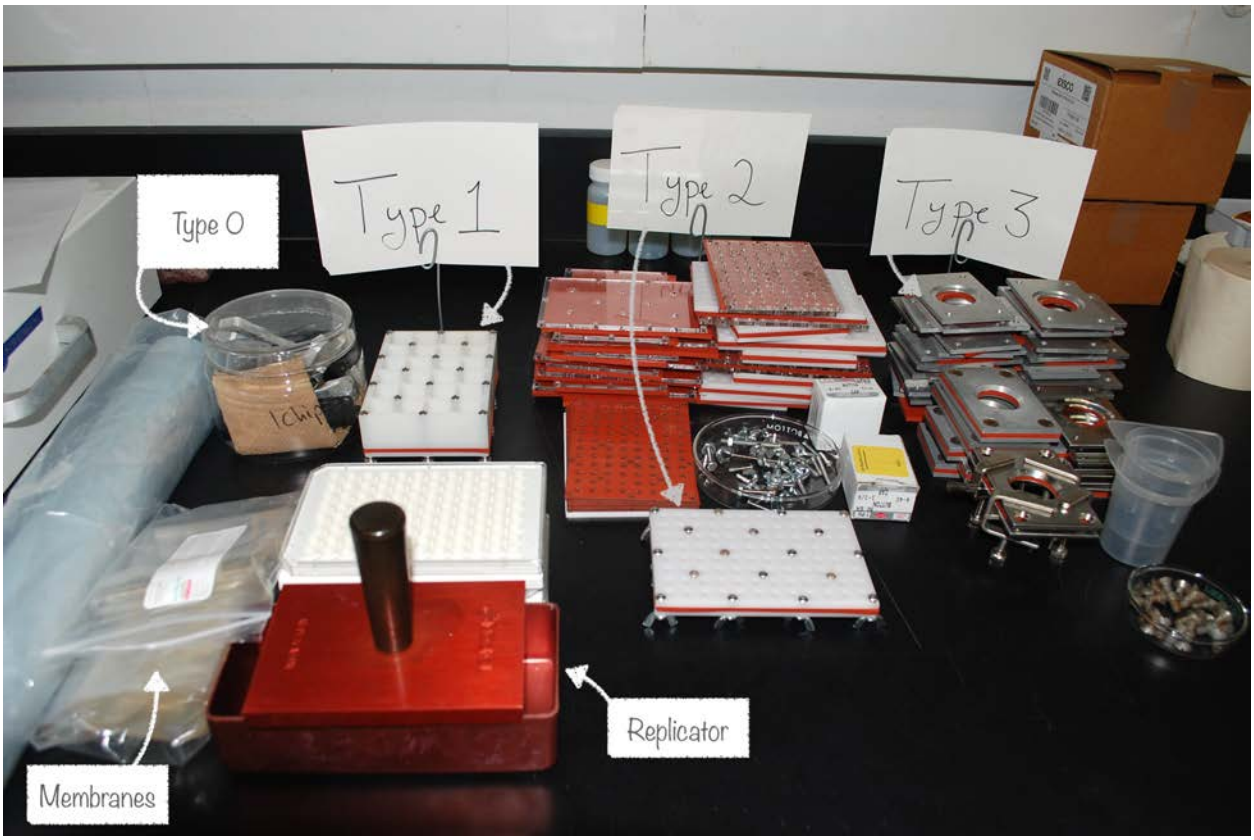


Figure 1.1: Types 0 ("Ichip"), 1, 2, and 3 ("Iplate Suite") designs of diffusion microchamber isolation-cultivation devices (Soil Ecology Laboratory, Department of Soil Science, UW-Madison).

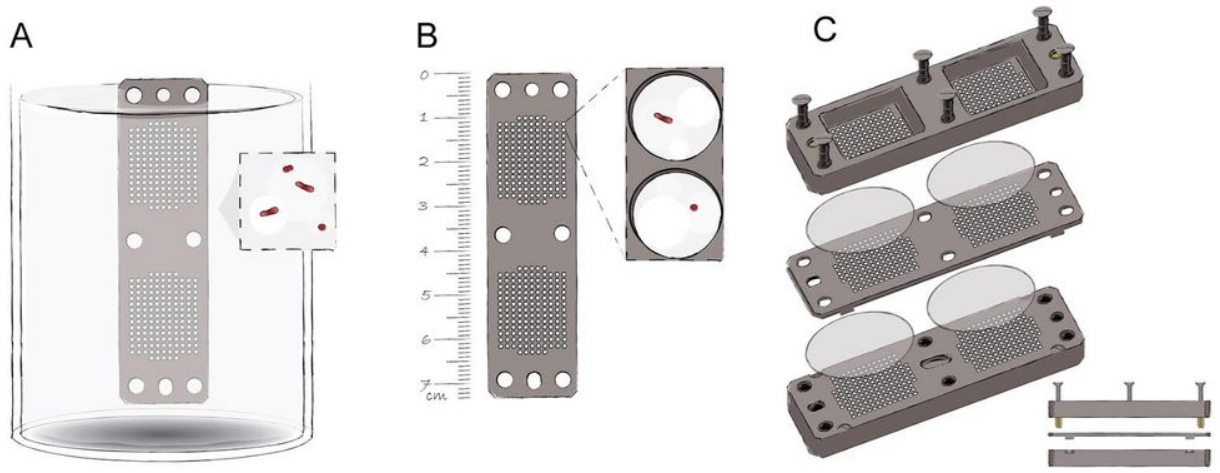


Figure 2.1: Ichip device parts and assembly presented by Nichols et al. (2010).

evolving to devices akin to Rose chambers (Type 3). There are many new and interesting ways to customize and adapt the design to fit any investigator's exact needs or system of study (water, soil, sediment, etc.), and the sourcecode for laser-cutting operation and 3D-printing will be made available in a forthcoming publication and repository. The protocols of preparation of inocula to the desired cell density is left to the investigator, but a buffered nutrient solution is recommended. A note on post-incubation cell-detection is noted in the final section below, with a reminder that this project is intended to evolve and develop with novel input from those who give this a try.

Funding for this project was provided by the Wisconsin Institutes for Discovery and the UW-Madison Soil Ecology Laboratory. The Principle Investigator overseeing this project is Dr. Thea Whitman, with generous assitance by Annie Edwards, and if you have questions or recommendations, please email her or Michael J. Braus at `brausm@protonmail.com`.

2 Type 0: Original Ichip

The original Ichip is a device that was presented by Nichols et al. (2010). There are great advantages to using a diffusion-based system of isolating while cultivating microorganisms, but there are also problems with this device that inspired the creation of the various "Iplate" extensions of the technology. These problems include the following:

- the minute size of the plugs on/in which microbes grow,
- the density of plugs as they are arrayed on the device,
- inoperability with existing pipets, tubes, etc. in most life science laboratories
- unwieldy assembly and disassembly, esp. regarding contamination, and
- unaffordable manufacture or customization for most investigators.

Four Ichip devices are available for use, and users are invited to assess for them-

selves the advantages and disadvantages of this design.

2.1 Inventory

Devices Available: 4 (as of 2020-01-01)

- Thin black polyethylene glycol (PEG) "middle plate" with 2 side-by-side arrays of 179 through-holes each.
- Aluminum housings ("upper and lower plates") for containing the membrane-bound plugs.
- One roll of thin plastic membrane (non-sterile).
- Hardware of bolts (the upper and lower plate housing has internal threaded bolts).
- Dimensions when assembled: 1.4 cm x 7.2 cm x 2.4 cm.

2.2 Protocol(s)

1. Sterilize all parts of Ichip with a combination of autoclaving, alcohol, flame, and/or UV.
2. Inoculate warm agar (as cool as possible but still molten/liquid) with bacteria or other cells such that there is rouhgly 1 cell per volume of each microchamber.
3. Dip central plate into agar, let cool a moment, and scrape away excess to leave the surface of plugs in the through-holes flush.
4. Apply membrane to each side of the central plate.
5. Place the membrane-bound central plate in the upper and lower plate housing.
6. Insert and tighten screws with sufficient pressure to prevent cross-flow between microchambers.
7. Bury in a pot of soil/sediment in the laboratory/incubator or the field for any desired length of time.
8. Disassemble the device in reverse order, being careful to prevent cross-contamination between chambers. This can be assisted by using clips to hold the membranes flat to the central plate while it dries, but it this does not guarantee zero contamination.
9. Use the tip of a straigthened paper clip to push the plugs out of the central plate into a tube or well-plate for analysis or resuspension for another incubation.
10. Clean and refurbish the metal and plastic parts of the device for storage and later use.

3 Type 1: "Deep-96" Iplate

The "Deep-96" Iplate device suspends a bolus of media (liquid, solid, gas, colloid, suspension, etc.) above 4 microchambers below, with which the plugs and

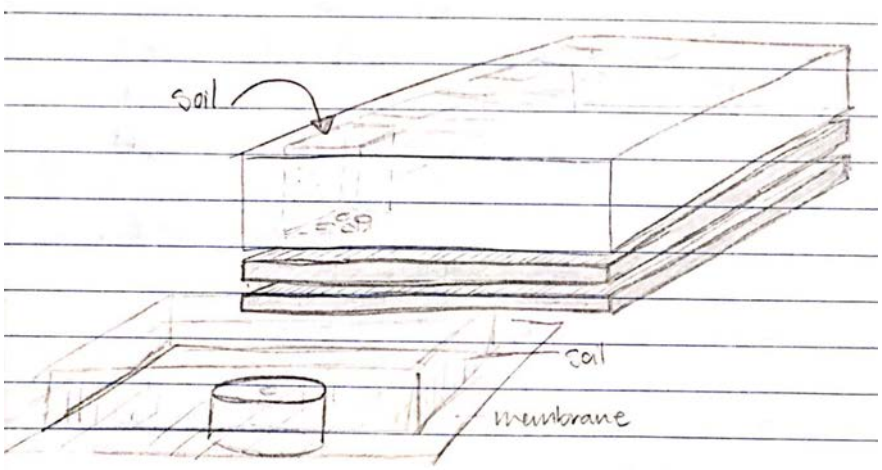


Figure 3.1: "Deep-96" Iplate device parts and assembly. Note the 4 chambers housed in each "well". Sketch by Annie Edwards.

3.1 Inventory

Devices Available: 1 (as of 2020-01-01)

- Upper plate: Laser 3D-printed plastic with polished bottom.
- Middle plate: Delrin or silicone.
- Lower plate: Delrin.
- Membrane: Cellulose dialysis tubing or plastic nuclear pore.
- Hardware of long bolts and thumb-nuts or wing-nuts.
- Through-holes dimensions: thickness of $1/8$" (3.175 mm) and diameter of 3.0 mm.
- Dimensions assembled: 11.5 cm x 3.5 cm x 7.75 cm.
- Microchamber volume $= \pi r^2 h$, where $h = 3.175$mm and $r = 1.5$cm $\rightarrow 22.4\mu$L. However, the laser cutter causes a slight cone-shape instead of a perfect cylinder as the melted Delrin slightly pools as it cools, so the real volume may be %5 to %10 smaller. 20μL worked very well throughout our tests.

3.2 Protocol(s)

3.2.1 Assembly & Incubation

1. Sterilize the plates, hardware, and membranes using a combination of autoclaving, flame, soaking in alcohol, or UV treatment (the latter works well on the membrane).
2. Partially assemble the Iplate using the lower plates that have screw-holes but no through-holes, one sheet of membrane, and the central plate. This can be held together with strong clips, clamps, or with the nuts and bolts.
3. Pipet sterile molten agar or other gel medium, with or without added nutrients or buffers, into the open through-holes of the central plate.
4. Remove the lower plate and replace with a lower plate that has through-holes, then pipet dilute inoculum onto the top of the resulting plugs situated

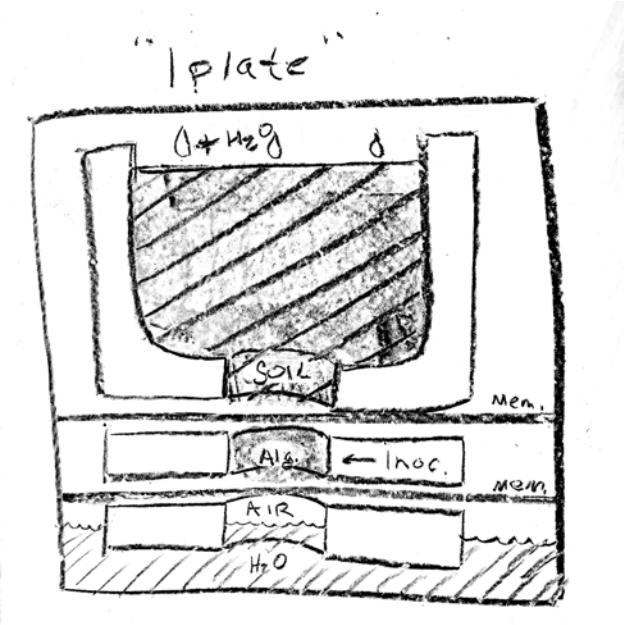


Figure 3.2: Simplified sketch of Iplate device. "Alg." signfies either alginate, agar, or any medium of choice for the intermembrane plug that is inoculated. Chalkboard (inverted color) by Michael Braus.

in the central plate.

5. Apply a membrane to the central plate and finally the "deep-well" upper plate with partitioned through-holes to the assembly, inserting its longer bolts to tighten with nuts (wing-nuts work fine).

Figure 3.3: Potting soil (e.g. Sungro) that is very good for use with the Ichip or Iplate devices. This kind of soil is very high in organic matter (earthy brown material) and large mineral fertilizer (silvery particles).Adding this to a more mineral-rich field soil would be wise, because field soil is populated with microorganisms that may improve the cutivability of the target organisms in the microchambers.

Figure 3.4: Applying water to assembled and loaded Type 1 “Deep-96” Iplate device. This assembly can then be wrapped in parafilm or put in a cabinet or incubator.

3.2.2 Disassembly

The following figures demonstrate a successful technique for disassembling an Iplate device after the incubation period.

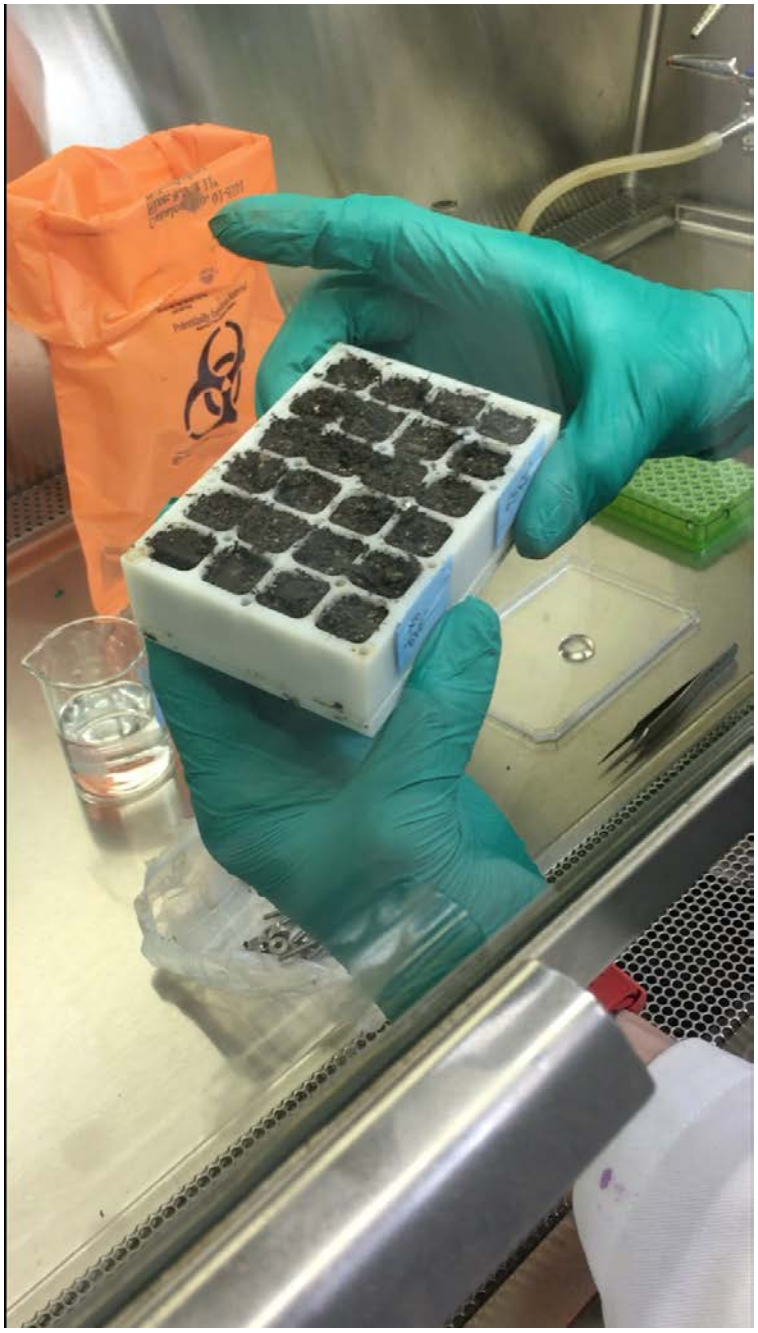


Figure 3.5: DISSASSEMBLY STEP 1. Remove the device from its incubation cabinet, any parafilm covering it, and all hardware. Keep the plates flush and apply a little pressure with your fingers or clips/clamps as it is disassembled.

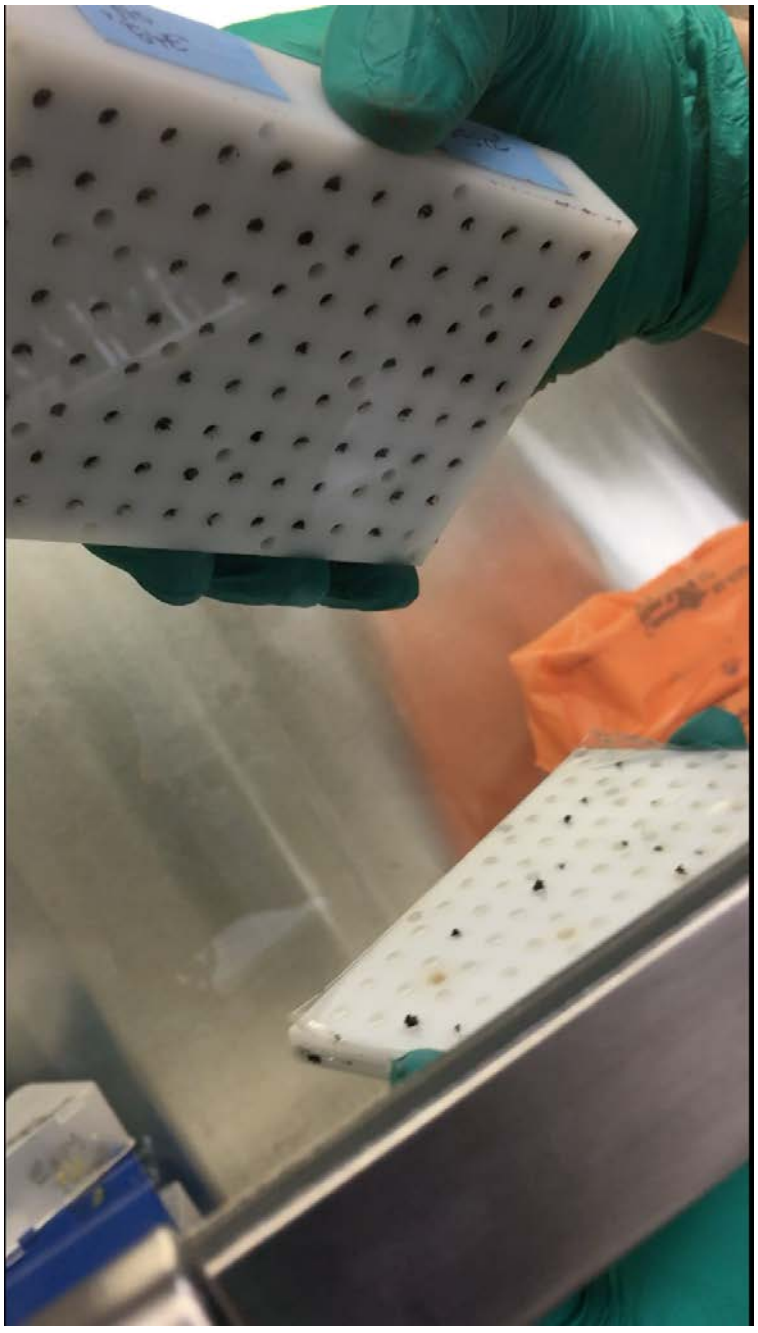


Figure 3.6: DISSASSEMBLY STEP 2. Keeping the upper membrane flattened to the top of the middle plate, remove the upper plate (96-well with deep pools).

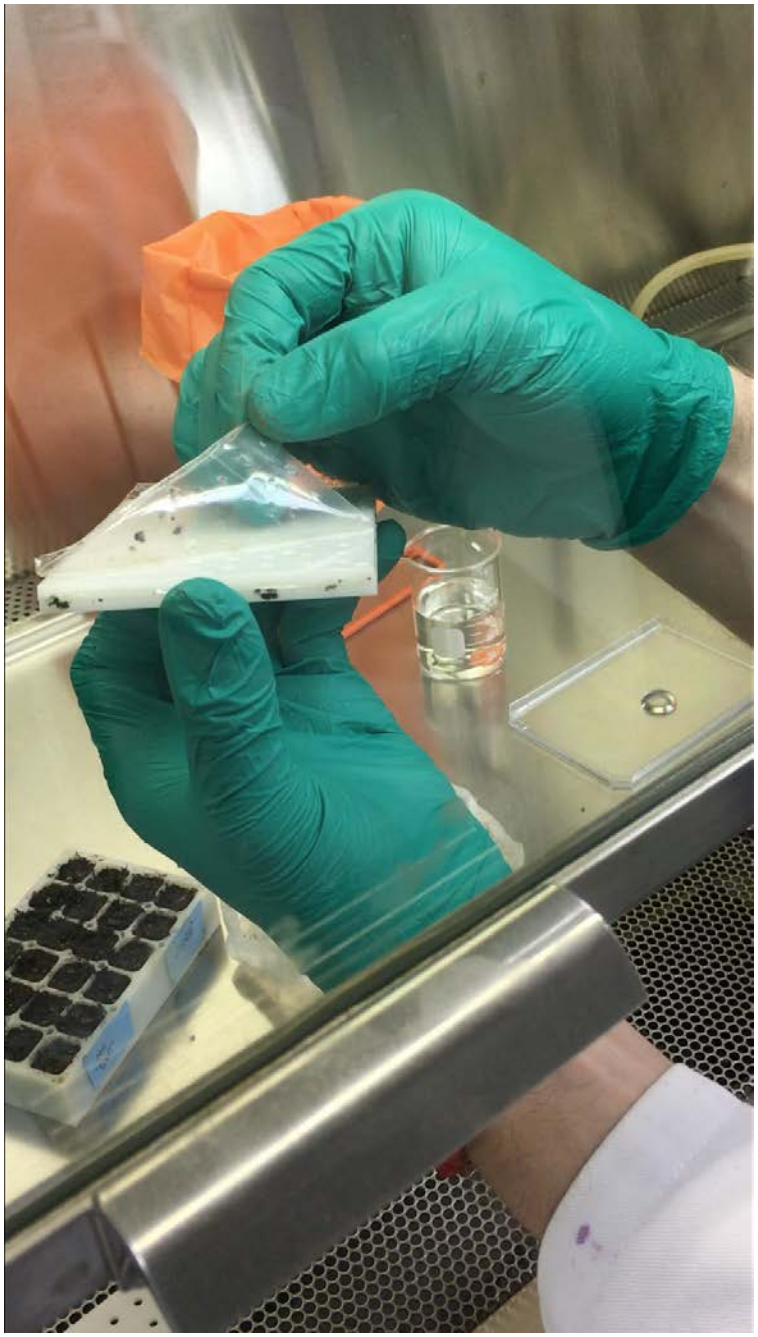


Figure 3.7: DISSASSEMBLY STEP 3. Peel away the membrane from the upper side of the middle plate. You need to allow these membranes to dry a little to prevent the "flowing miniscus" that travels across the plate as the membrane is peeled away. This can cause significant contamination, which makes this the most crucial step in the protocol, especially if the chambers of this plate each have different isolates.

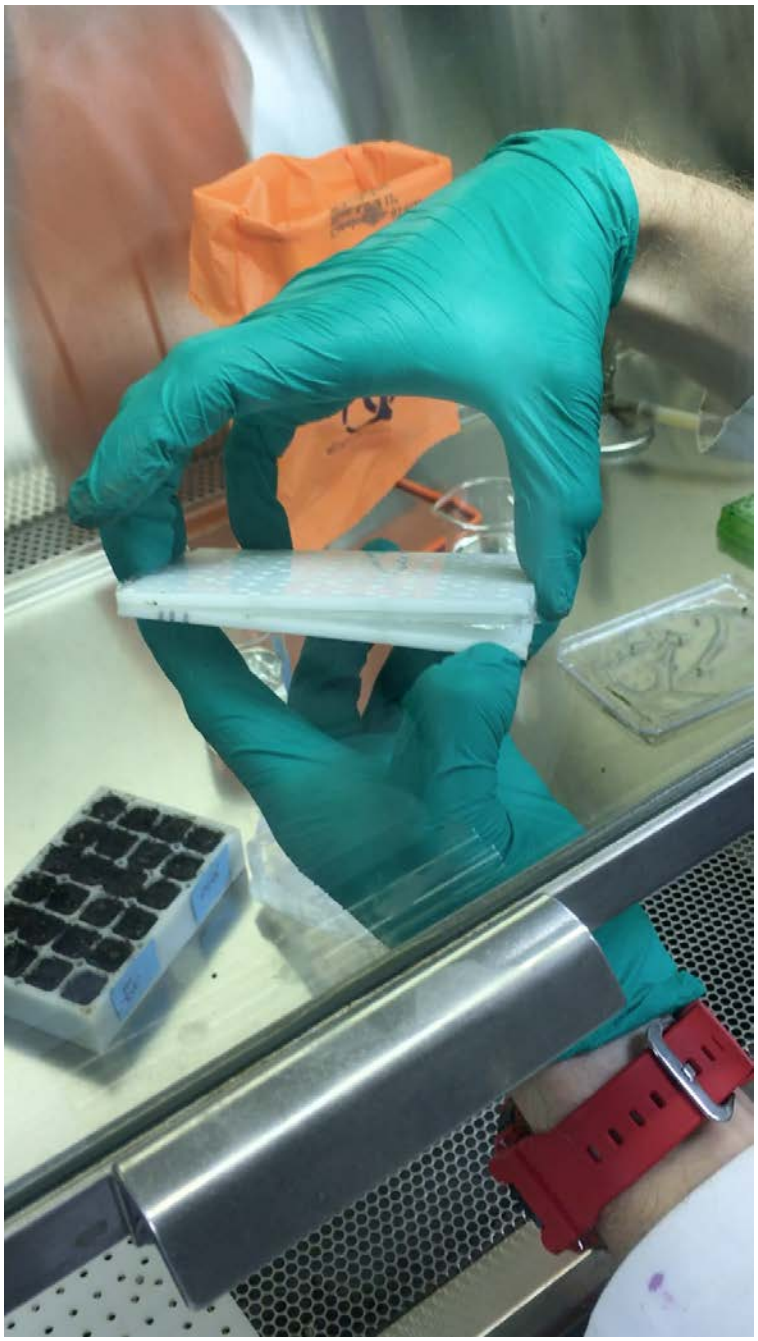


Figure 3.8: DISSASSEMBLY STEP 4. Remove the lower plate from the middle plate while keeping the membrane flattened to the bottom of the middle plate.

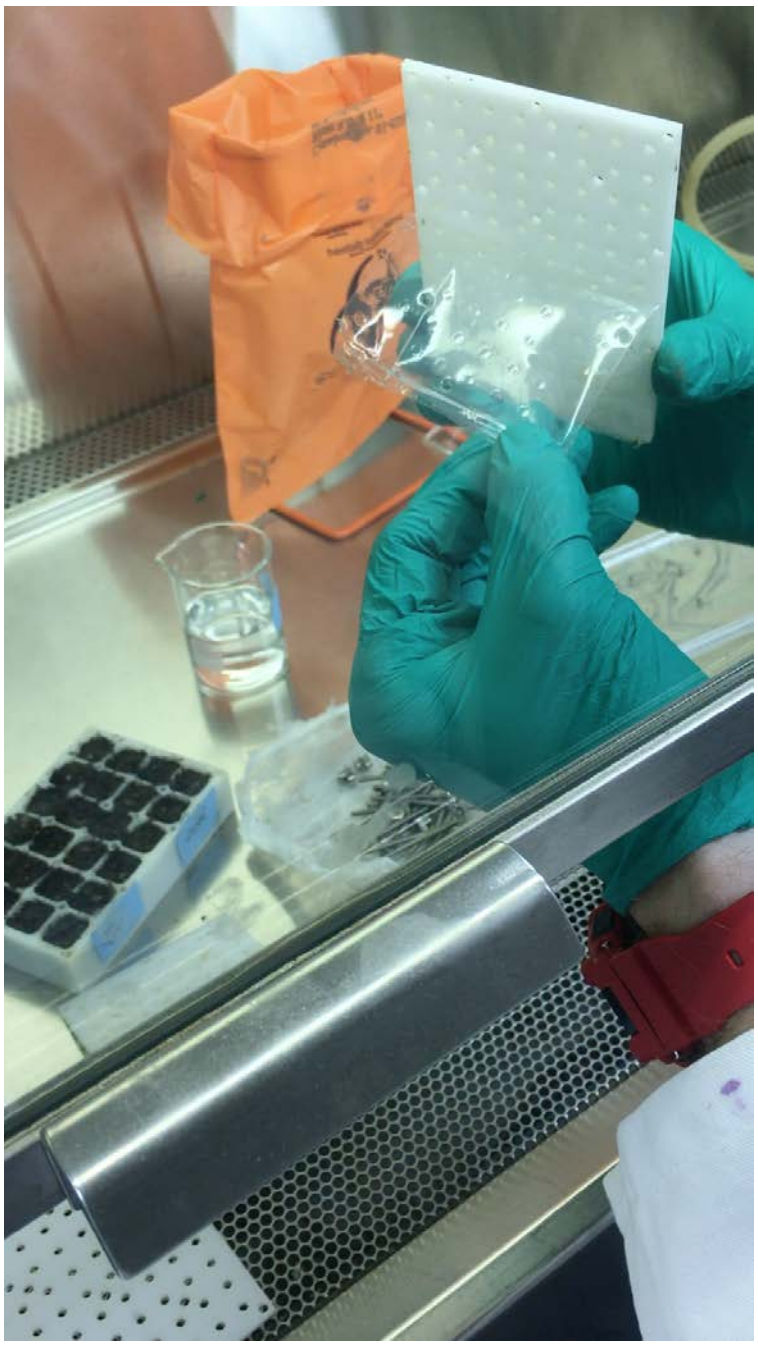


Figure 3.9: DISSASSEMBLY STEP 5. Peel away the membrane from the bottom of the middle plate, first allowing the membrane to dry a little to prevent the “flowing miniscus”.

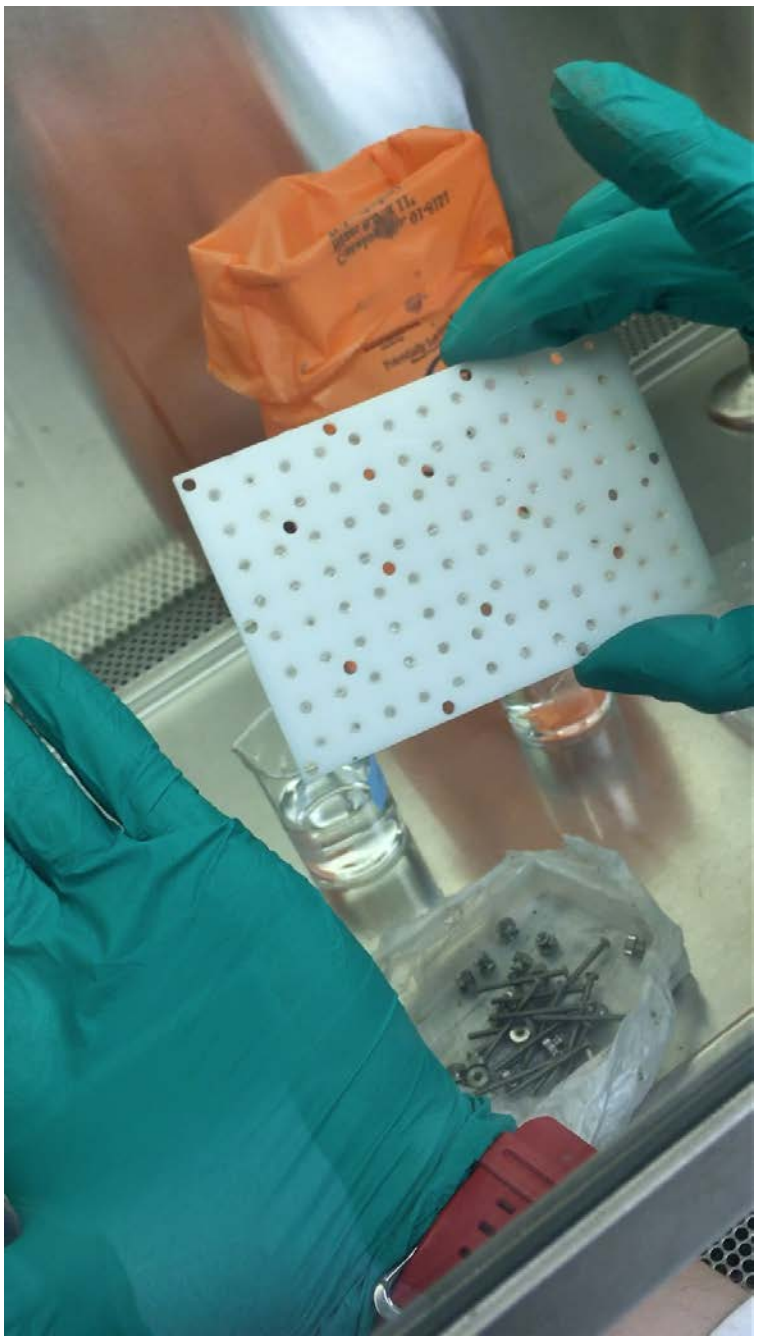


Figure 3.10: DISSASSEMBLY STEP 6. Check that the plugs are intact, and note whether any plugs have dried out or are mishapen from intermittent drying during incubation.

4 Type 2: "Flat-96" Iplate

4.1 Inventory

Devices Available: 10 (as of 2020-01-01)

- Upper plate: Laser 3D-printed plastic with polished bottom.
- Middle plate: Delrin or silicone.
- Lower plate: Delrin.
- Membrane: Cellulose dialysis tubing or plastic nuclear pore.
- Hardware of long bolts and thumb-nuts or wing-nuts.
- Through-holes dimensions: thickness of 1/8" (3.175 mm) and diameter of 3.0 mm.
- Dimensions assembled: 3/8" (9.525 mm) x 11.5 cm x 7.75 cm.
- The volume of the microchambers is the same as the "deep-well" plate (specs listed above in that section) because the silicone middle plate is identical here.

4.2 Protocol(s)

This device is assembled and disassembled in a virtually identical manner to Type 1 (see above). This type cannot hold soil the way the "deep-well" Iplate can, but it is more adaptable to burying in porous media like soil or suspending in a liquid medium if desired. It is recommended, however, that the Type 2 Iplate be loaded into soil on a 45° angle, because the water that is added neither pools in the through-holes of the upper plate, as it would if loaded into soil horizontally, nor flows off entirely, as it would if the device were loaded into soil vertically.

5 Type 3: "Flat-Coin" Iplate

This device is an adaption of the "Rose chamber", which is a device used largely in phycology to observe the real-time growth or behavior of algae or protists. Originally, the upper and lower housing plates held a silicone sheet with a "coin" of material removed, and the central silicone plate was contained with large glass cover slips. These glass parts have a tendency to crack, so the pressure applied only gently holds the system together. Once one removes the glass and replaces this with a membrane, such as dialysis tubing, for use as a diffusion chamber, the "bugs" can be grown inside the chamber with the soil or medium on the outside, or the bugs can be grown outside the chamber with the soil or medium on the inside.

5.1 Inventory

Devices Available: 25 (as of 2020-01-01)

- Upper and lower plates: steel or aluminum $\approx$ 1/8" (3.175 mm).
- Central plate: silicone with thickness of 1/8" (3.175 mm).

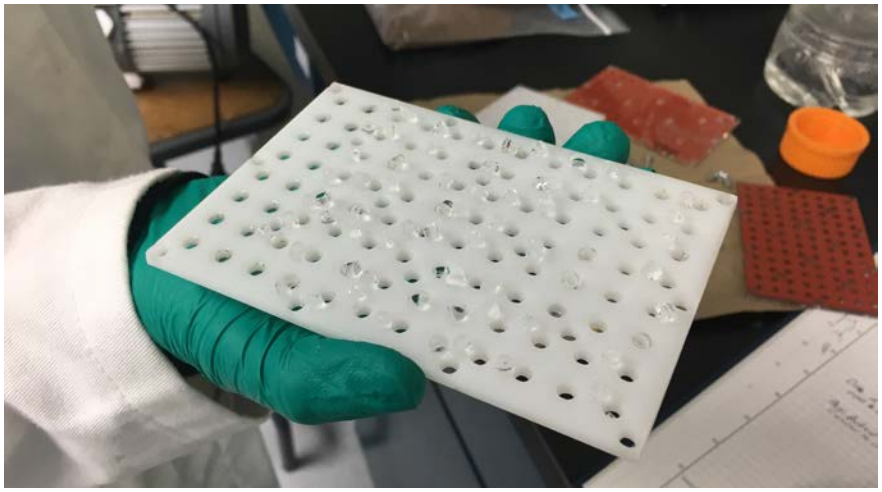


Figure 4.1: A test of agar plug shape, size, and durability when removed or transported from the central plate of the Type 2 Iplate ("Flat-96"). When loaded with agar, this design allows for the use of a multipipet and produces plugs of an optimal size for inoculation and subsequent manipulation, microscopy, etc.

- Dimensions assembled: 7.5 cm x 5.0 cm x 0.9 cm.
- The volume of the internal chamber is $\pi r^2 h$ where $h = 3.175$ mm and $r = 1.25$ cm $\rightarrow$ 1.6 mL.

5.2 Protocol(s): Soil Inside, Bugs Outside

1. Assemble the lower plate, membrane, and middle plate with the nuts and bolts (or clips/clamps). A washer may help as the head of the bolt is directly on the surface of the silicone middle plate.
2. Load soil into the coin-shaped hollow of the middle plate, gently tamping it down. It helps to partially wet the soil before loading, which also prevents clays and silts from blowing onto the outer surface of the middle plate, which can cause problems for later sealing via pressure from the upper membrane and upper plate.
3. Remove hardware (or clips/clamps if you used those instead) and apply another membrane and upper plate.
4. Insert and tighten the nuts and bolts such that the internal chamber is sealed from its exterior environment.
5. Inoculate the top and/or bottom of the "coin" of soil. Streaking may be possible as well, just as one would on the gel of a Petri dish. Alternatively, a thin inoculated gel may be applied to the surface instead, which would allow for the diffusion of growth factors from the soil to the gel, but this is not guaranteed to function because the diffusion will be very, very slow (see Fick's law).
6. Incubate in a humid space and alternately wet the surface if it appears to dry out. Some soils can be very absorbent, even after initial wetting to help load the soil into the device.

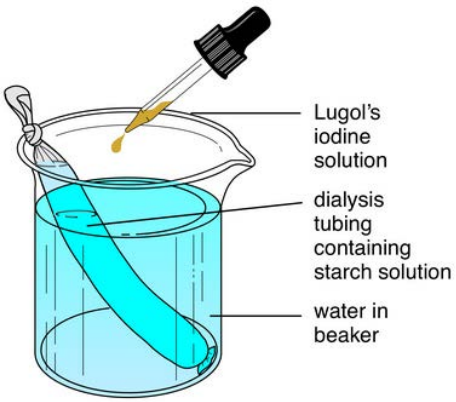


Figure 6.1: Lugol's iodine carbohydrate test to distinguish the variable diffusion of polysaccharides versus monosaccharides across a cellulosic dialysis tubing membrane. This experiment inspired the use of dialysis tubing for the Ichip/Iplate membrane diffusion of growth factors while disallowing cells to transfer between the inside and outside of microchambers.

5.3 Protocol(s): Bugs Inside, Soil Outside

1. Assemble the lower plate, membrane, and middle plate with the nuts and bolts (or clips/clamps). A washer may help as the head of the bolt is directly on the surface of the silicone middle plate.
2. Load liquid/molten agar into the empty space, slightly overfilled, and allow to cool. Scrape across with a clean scalpel such that the gel surface is flush with the middle plate's upper surface.
3. Inoculate the top and/or bottom of the "coin" of gel. Streaking may be possible as well, just as one would on the gel of a Petri dish.
4. Remove hardware (or clips/clamps if you used those instead) and apply another membrane and upper plate.
5. Insert and tighten the nuts and bolts such that the internal chamber is sealed from its exterior environment.
6. Incubate buried in soil or with soil piled on top, and alternately wet this soil if it appears to dry out.

6 Raw Materials Tests & Specifications

6.1 Membranes

The use of membranes to facilitate diffusion microchamber was inspired by such experiments as Lugol's carbohydrate test. In this test, the membrane allows for the passage of a specific-sized molecule to travel between the inside and outside of a sealed tube of dialysis membrane sitting in a solution to which the analytical test solution is added.

The simplest and most affordable membrane is standard medical-grade dialysis tubing, which is often $\approx$ 10 mm in width (varies with saturation) and 6.4 cm in diameter of variable length, and it is sold dry and flattened in packages of

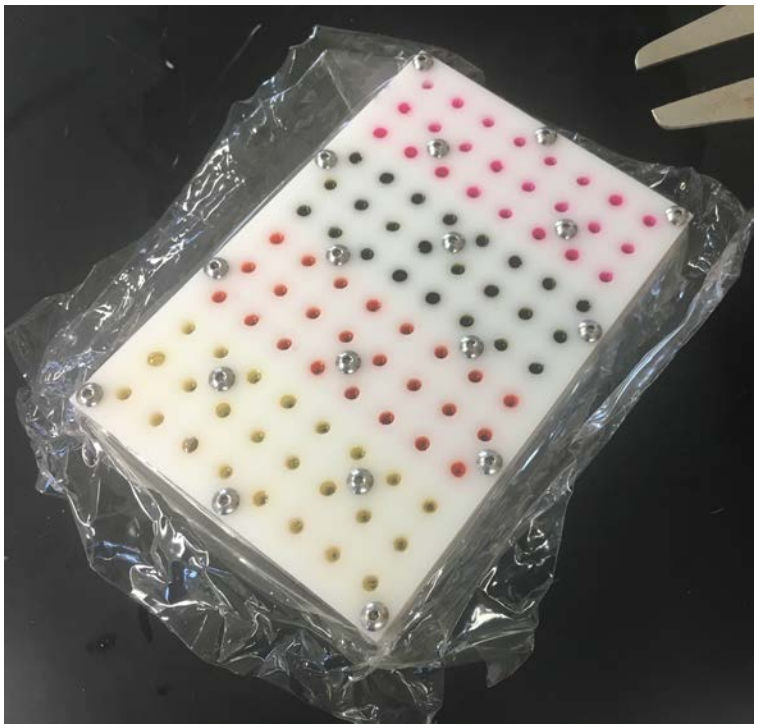


Figure 6.2: Flux test of membrane permeability and absorption by membrane using common dyes, part 1 (assembled). Dyes Key: Yellow = Iodine, Red = Congo Red, Dark Blue = Thymol Blue, Pink = Rose Bengal.

3 m for less than $10. This material is a highly purified crystalline cellulose-based membrane, which when dry acts like a tough plastic sheet and when wet is a soft and pliable (but not stetchable) film. The drying and wetting of the membrane causes virtually no change to its behavior, but the material is easily stained by high-charge-density compounds, suggesting that it will absorb some of the dilute growth factors in soil solution. Rose bengal (MW = 974 Da) and thymol blue (MW = 466 Da) appeared to flow through the cellulosic membrane better than congo red (MW = 697 Da) or iodine (MW = 126 Da), further suggesting the strong sorptive abilities of the membrane are as influential as the molecular weight of the substance to determine what flows through this membrane. The membrane, when it is chemically saturated, allows the passage of molecules that are approximately < 14kDa, which would include vitamins, disaccharides, very small complex carbohydrates, amino acids, small peptides, nucleic acids, and small RNA/DNA. It is unknow the extent to which lipids or fats are passable as well.

Specifications: Aldon Corp or Ward's Science+ Dialysis Tubing (75 mm x 10 feet, diameter 47.7 mm, LOT AD-17157); one may also try "SnakeSkin" dialysis tubing.

6.2 Other Raw Materials

- Sheet(s) of Delrin: 1/8"
- Sheet(s) of silicone: 1/8"
- Bolts: 4-40, 9/16 button cap.

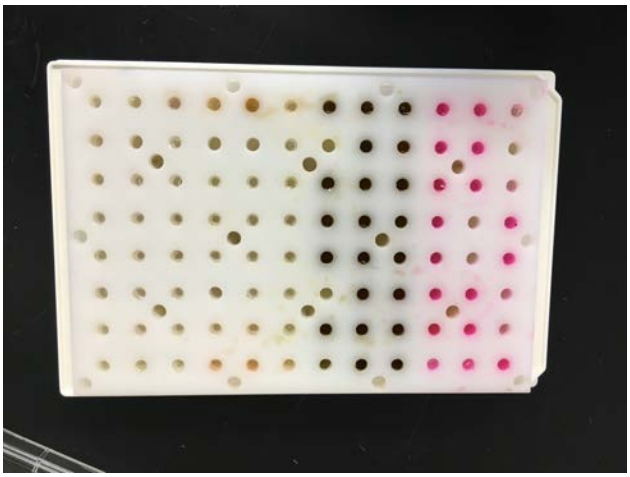


Figure 6.3: Flux test of membrane permeability and absorption using common dyes, part 2 (disassembled). The plugs show the color of the dye that passed through the membrane. Dyes Key: Yellow = Iodine, Red = Congo Red, Dark Blue = Thymol Blue, Pink = Rose Bengal.

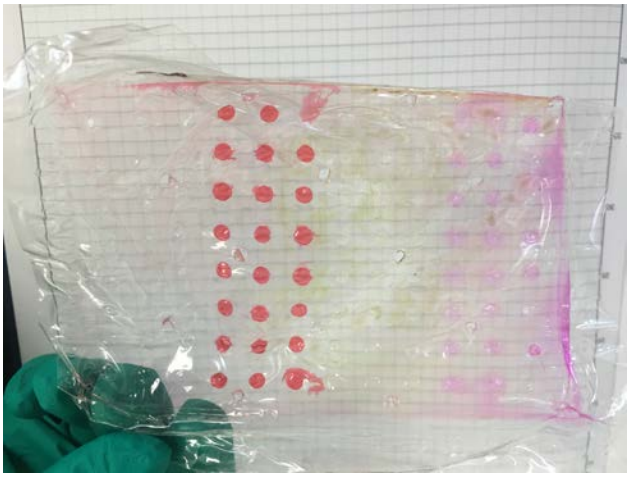


Figure 6.4: Flux test of membrane permeability and absorption using common dyes, part 3 (residue analysis). The membrane also absorbed a portion of the dye applied. Dyes Key (left to right): Yellow = Iodine, Dark Blue = Thymol Blue, Red = Congo Red, Pink = Rose Bengal.

Figure 6.5: Sheets of Delrin and silicone with simple hardware, all very inexpensive.

- Nuts (button, wing, thumb, etc.)
- Boekel 96-well plate replicator (“frogger”)

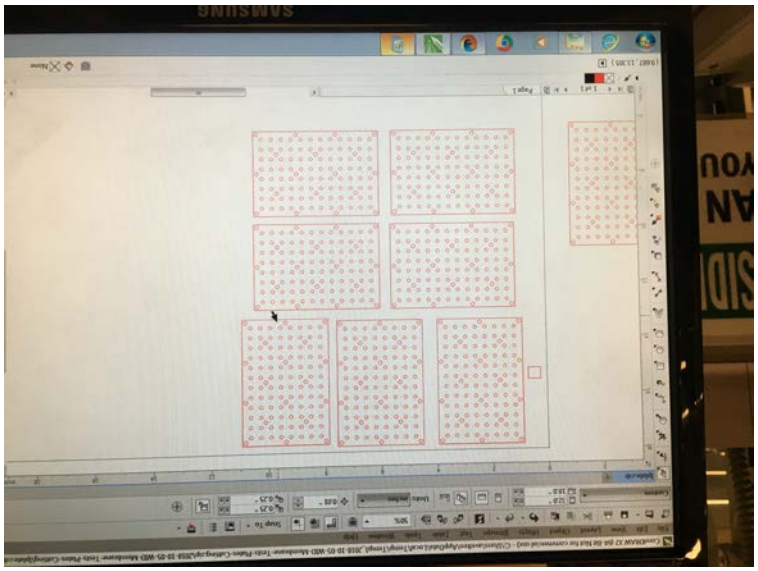


Figure 6.6: Operation of laser cutter to cut Delrin sheets to 96-through-hole plates.

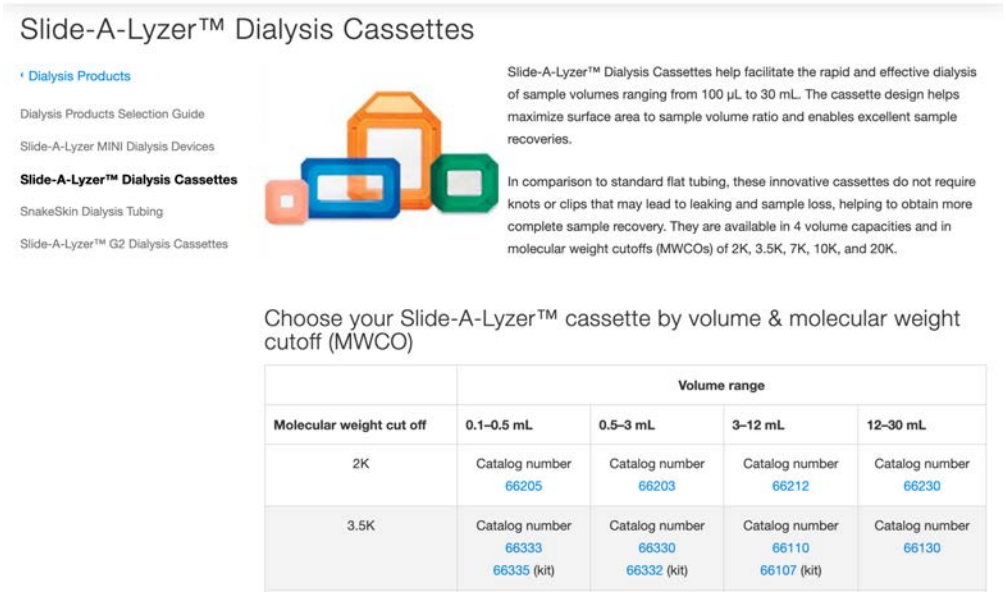
7 Co-Opting of Other Hardware and Equipment

There remain many possible pre-made hardware and equipment in the laboratory to function as diffusion microchambers. One such example is the "Slide-A-Lyzer" dialysis cassette. The "molecular weight cutoff", meaning the maximum molecular weight of molecules allowable across the membrane, can be selected from 2 kilodaltons to 20 kilodaltons. The chamber inside is loaded with any material, even a gel if one keeps the whole system warm, via a syringe inserted into one of the septa situated in the corners. Likewise, sterile gel may be loaded into the chamber and then may be inoculated with another syringe after cooling. If one is delicate, cells can be spead somewhat evenly across the membrane just inside the chamber, which, when buried or added to a soil or liquid medium, will receiv the highest density of diffusing growth factors. One may also inject a soil slurry into the chamber and growth microbes on the surface of the membrane, which (we must assume) is cellulosic.

Roughly 6 cassettes can be purchased for $\$135$. Following the overall philosophy of this project, one can be very inventive with simple materials to recreate and simulate the complex microenvironments in which soil bacteria and other microorganisms grow.

8 Post-Incubation Cell Detection

There are many existing methods for detecting and identifying microbes: DAPI, cytometry, FISH, etc. The detection of microbes cultured and isolated with the materials and methods above will be left entirely to the investigator. However, the designers and testers of the "Iplate Suite" encourage users to be open-minded and creative at every stage, thinking 4-dimensionally, empathizing at all times



Slide-A-Lyzer™ Dialysis Cassettes

‹ Dialysis Products

Dialysis Products Selection Guide

Slide-A-Lyzer MINI Dialysis Devices

Slide-A-Lyzer™ Dialysis Cassettes

SnakeSkin Dialysis Tubing

Slide-A-Lyzer™ G2 Dialysis Cassettes

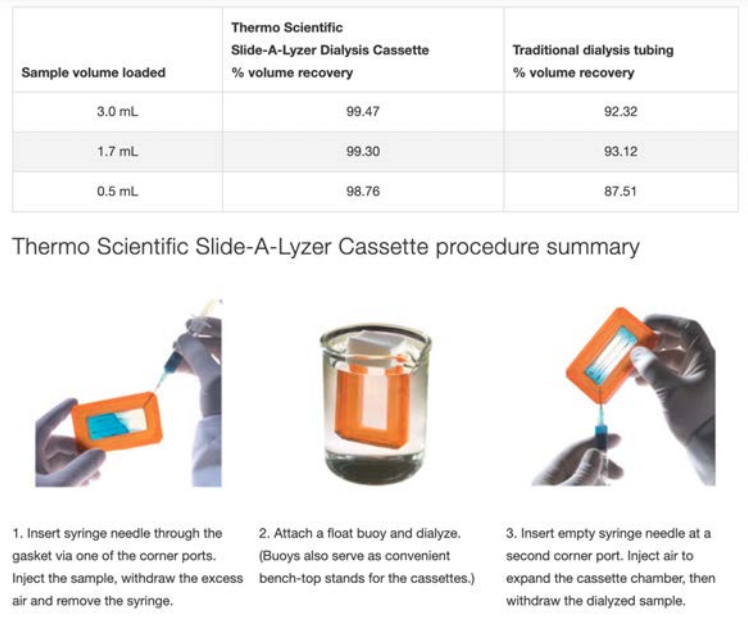
Slide-A-Lyzer™ Dialysis Cassettes help facilitate the rapid and effective dialysis of sample volumes ranging from 100 µL to 30 mL. The cassette design helps maximize surface area to sample volume ratio and enables excellent sample recoveries.

In comparison to standard flat tubing, these innovative cassettes do not require knots or clips that may lead to leaking and sample loss, helping to obtain more complete sample recovery. They are available in 4 volume capacities and in molecular weight cutoffs (MWCOs) of 2K, 3.5K, 7K, 10K, and 20K.

Choose your Slide-A-Lyzer™ cassette by volume & molecular weight cutoff (MWCO)

	Volume range			
Molecular weight cut off	**0.1–0.5 mL**	**0.5–3 mL**	**3–12 mL**	**12–30 mL**
2K	Catalog number 66205	Catalog number 66203	Catalog number 66212	Catalog number 66230
3.5K	Catalog number 66333 66335 (kit)	Catalog number 66330 66332 (kit)	Catalog number 66110 66107 (kit)	Catalog number 66130

Figure 7.1: Source: https://www.thermofisher.com



Sample volume loaded	Thermo Scientific Slide-A-Lyzer Dialysis Cassette % volume recovery	Traditional dialysis tubing % volume recovery
3.0 mL	99.47	92.32
1.7 mL	99.30	93.12
0.5 mL	98.76	87.51

Thermo Scientific Slide-A-Lyzer Cassette procedure summary

1. Insert syringe needle through the gasket via one of the corner ports. Inject the sample, withdraw the excess air and remove the syringe.

2. Attach a float buoy and dialyze. (Buoys also serve as convenient bench-top stands for the cassettes.)

3. Insert empty syringe needle at a second corner port. Inject air to expand the cassette chamber, then withdraw the dialyzed sample.

Figure 7.2: Source: https://www.thermofisher.com

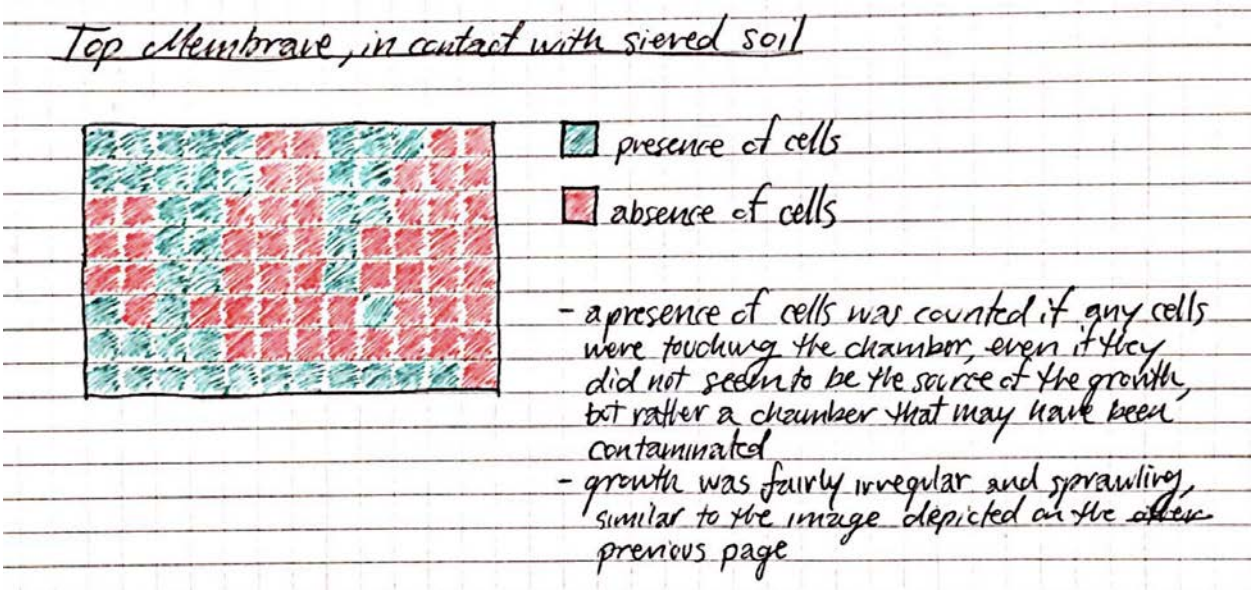


Figure 8.1: An example of creactive "4-dimensional thinking": the detection of cells was performed on a membrane instead of on the agar plugs themselves of an incubation using the Type 2 Iplate device in potting soil. Idea and sketch by Annie Edwards.

with their target microorganisms. For example, perhaps the detection of cells should be performed on the membrane instead of the agar plugs, because the cellulosic membranes indeed provide a source of glucose to cellulose-degrading microorganisms. Perhaps the gel used in the intermembrane space should be alginate instead of agar when cultivating protists, such that they may more easily move or swim. Perhaps a "contamination" does not ruin the incubation but instead leads to an unexpected discovery, and so on.

9 Further Reading

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