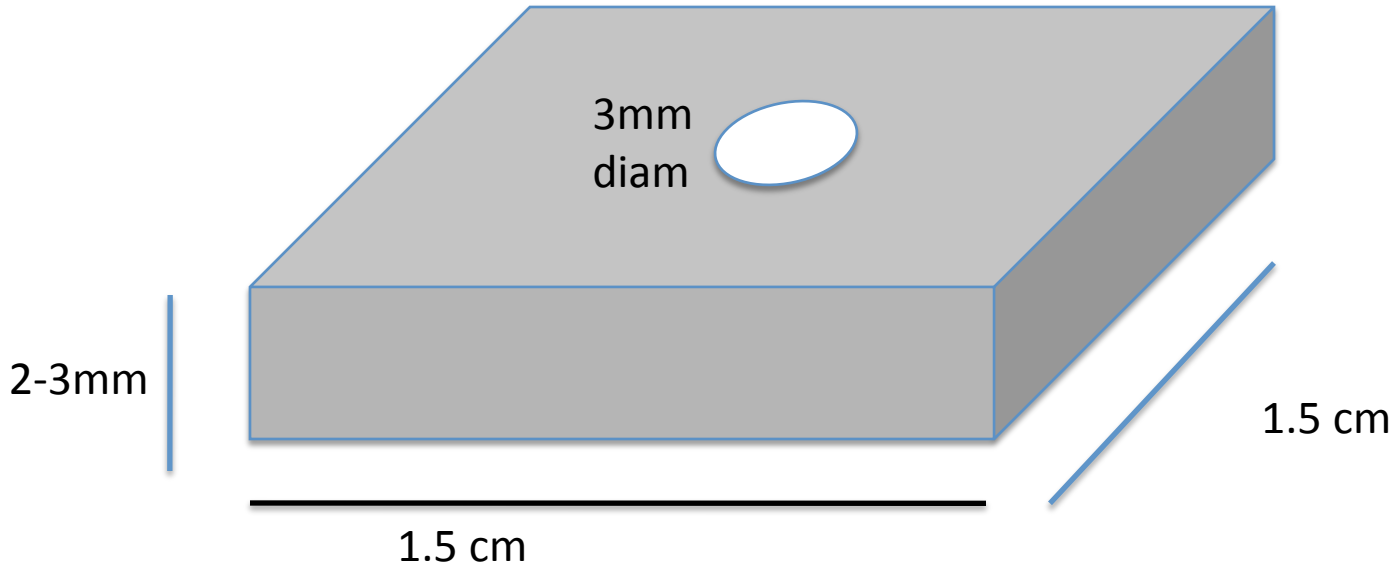
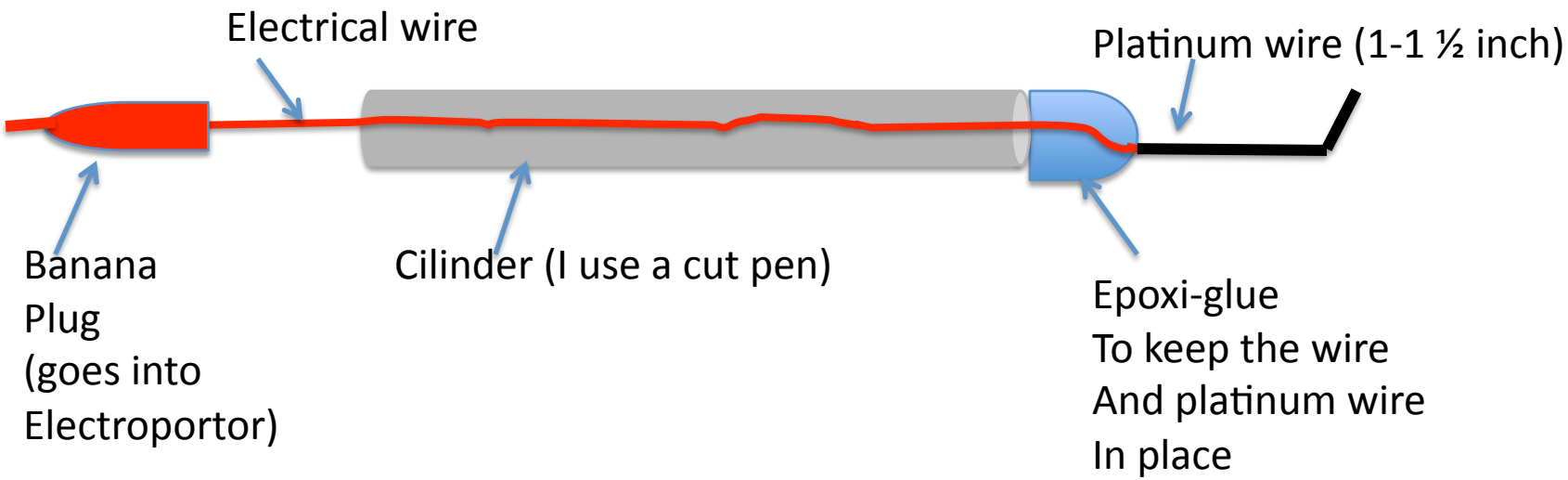
Electroportion chamber

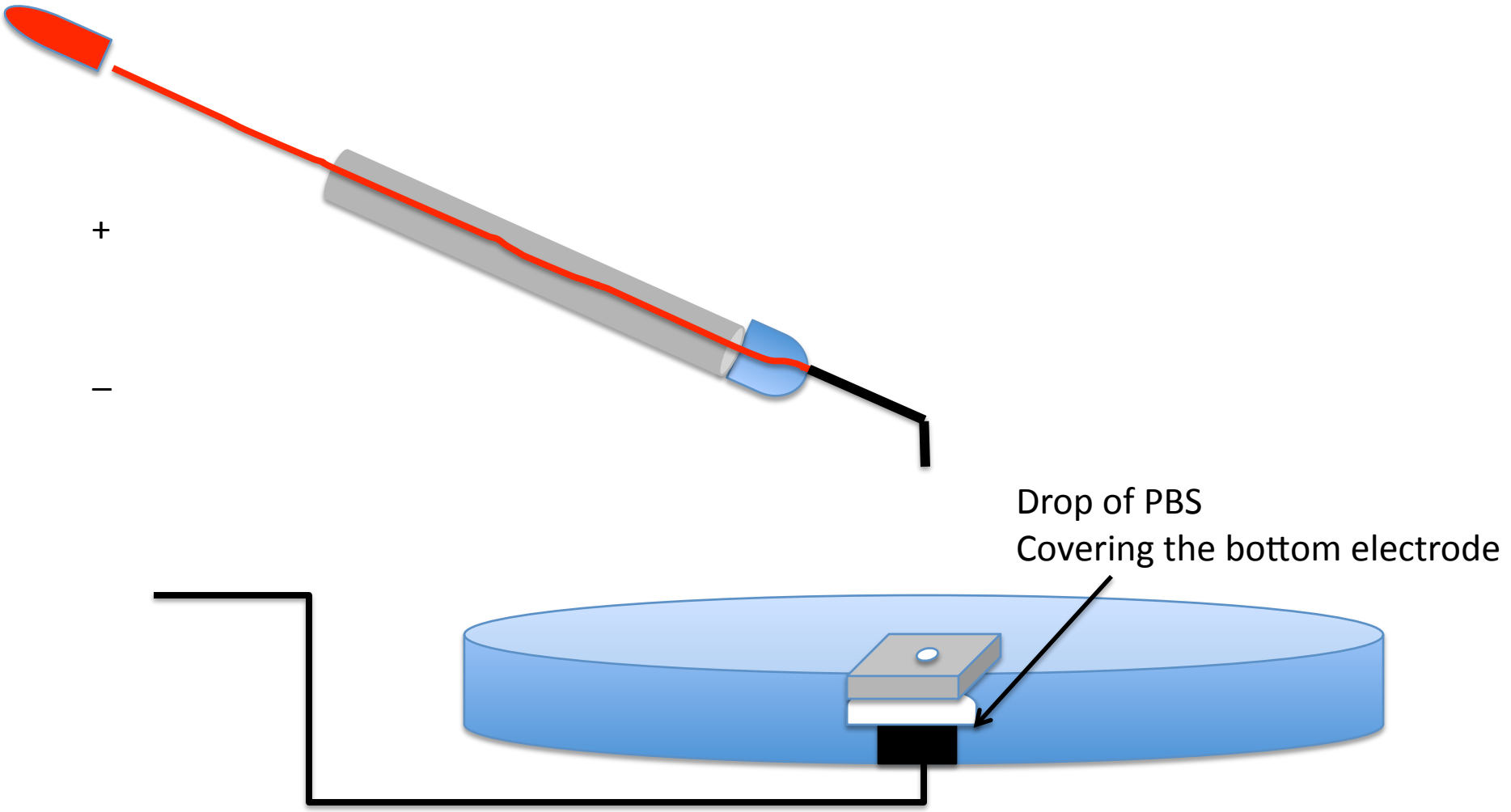


1. Made in plexiglass according to these measurements (I used to have them a little smaller, 1x1cm, but this size is more convenient)
2. Glue a square piece of dialysis membrane to one side of the chamber, using silicone sealant (you can get it in any hardware store or aquariums)
3. Dialysis membrane: www.spectrapor.com , cat # 132103. It's a roll of tubing. What I did is cut the tube open and cut the piece of membrane from it . There might be better ways of doing it, and I'm not sure you even need to open the tubbing.
4. With use the membrane breaks….just peel it off and glue another piece.

Top electrode



1. Pass the electrical wire through the body of a pen (or any plastic cilinder, you can even use a cut down plastic pipette
2. Sutter a bannana plug on one end of the wire, and a platinum wire to the other end. The diameter of the platinum wire is not very important, but not too thin that it will break nor too thick….
3. Fill the platinum end of the pippete with epoxi-glue so that the platinum wire is held in place and doesn't move or jiggle around.


+
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Drop of PBS
Covering the bottom electrode

Embryonic cochlea electroporation

Materials:

-DNA to be electroporated: at a concentration between 1-3 ug/ul. I dilute it in ddH2O
-30% sucrose in PBS or H20 with a spatula's tip of fast green. Fast green is not necessary but it helps visualize the incorporation of DNA into the cochlea.
-Electroporation chambers: these were made by Dan Huynh in the engineering department. They are basically plastic squares with a hole drilled in the middle. At the bottom of each chamber there is a square of dialysis membrane attached. The membrane breaks after several uses but it's easily replaceable.
-Electrodes: the negative electrode is a chamber that connects via a cable to the electroporator. It's commercially available from Protech (www.Protechinternational.com). The positive electrode can also be bought from the same company, however, I had more success with the home made ones.

Preparation:

-Dissect the cochleas as usual. Take the inner ear from the embryos and incubate for 8 minutes in CMF-PBS with 1mg/ml dispase and 1mg/ml collagenase and allow them to recover for ~1hr in DMEM+10% heat inactivated serum. Dissect the cochleas out and keep in a Petri dish on ice with DMEM serum.
-Mix the DNA 1:1 to 1:5 with the 30% sucrose fast green solution.
-Assemble the electroporation setup: connect both electrodes to the electroporator. Set the electroporator to 25V, 50 ms. (the settings A through D correspond to pulse durations of 25, 50, 100 and 200 ms). Cover the bottom electrode with PBS and place the electroporation chamber membrane side down directly on top of the PBS covered electrode.
With a thin tip plastic transfer pipette, transfer 4 to 5 cochleas to the electroporation chamber. Remove excess medium and fill the chamber with DNA solution.
-Apply 8-10 pulses touching the top surface of the Dna solution with the positive electrode. I usually reverse the polarity of the current every 2 pulses to ensure maximum dna incorporation from all sides.
-Let the cochleas sit for about 20 seconds.
-Remove the cochleas with a trasfer pipette and wash in DMEM/serum before placing them on culture dish.

I culture the cochleas on 13mm SPI black membranes 1.0u (www.2spi.com, #B1013)in 4 well nunc plates, in DMEM+F2+Pennicillin (10ml DMEM +100ulF2+100ul 100xPen).
Depending on the construct, electroporated GFP should be visible after 6-10 hours of electroporation.