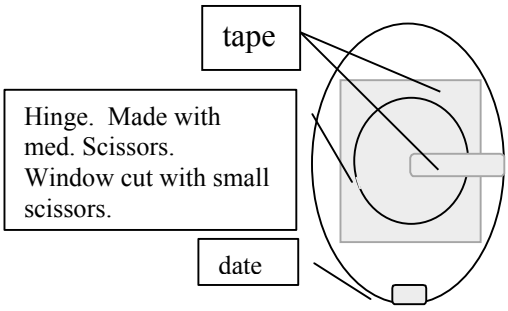
Egg Injection Techniques & Tricks

E2 injection techniques as developed by Karina Cramer and interpreted by Sue Hendricks.

Windowing Techniques

- **Set eggs in the morning. Use eggs ~52 hours after they were set (so, +4 hours 2 days later; stage 12-14). I get the best success with eggs that are set within a couple days of arrival (eggs arrive on Tuesday, so Thursday and Friday give me the best success).**
- **Bring up eggs on egg cardboard crate. Turn so that egg is on side**
- **Allow eggs to rest for ~10 minutes**
- **Swab liberally with 70% ETOH**
- **Wipe all instruments with 70% ETOH**
- **Cut Scotch clear plastic tape (Cat No. 191) ~ _ inch long for each egg**
- **Stretch tape and place centered on face of egg so that you have a large flat taped surface. You do not want wrinkles.**



- **Using medium sized scissors (blade length ~ _ " long), poke hole in round bottom part of egg (where the date is written, as opposed to the pointy top of the egg)**
- **With 3 cc syringe fitted with 18 _ g needle, withdraw 2.5-3 cc of albumin from egg. Angle needle down, so that you do not get any yolk. If you do get yolk, discard egg.**
- **With medium scissors, poke hole in lower left side of tape.**
- **With small scissors, make a hinged window a little bit smaller than a quarter. Try to have the scissors as parallel to shell surface as possible, to avoid breaking yolk.**
- **Cover hole made on end, with small square of plastic tape.**
- **Tape hinged window temporarily shut using a strip of plastic tape**

Approx. actual size

Injection Techniques

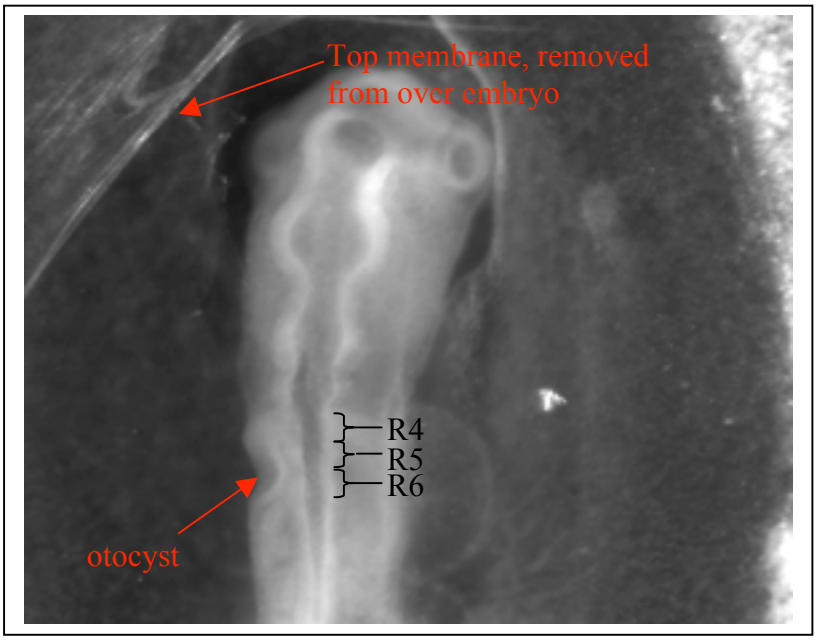
- **Turn on picospritzer, set to 10 ms pulses**
- **Turn on Fluorescence lamp if necessary**
- **Turn on air, set to 10 psi**
- **Fill pulled pipettes with dyes using Microfil Syring Needle**
    a. **Pull pipettes on David Kopf Vertical Pipette Puller Model 700C (downstairs) using the heater set @ 65, and solenoid set at 50.**
- **Place windowed egg on wax ring under dissection scope**
- **Open window, and use tape that kept it closed to keep it open**
- **Using your probe or fine forceps, pop any bubbles on surface of egg. Discard any egg in which the yolk has broken.**
- **You should be able to see the embryo (although it may take some practice) but to see it better, we inject India ink *under* the embryo**
- **India ink injection:**
    a. **1:30 dilution of store-bought stationary India ink in sterile PBS. We generally make 30 mls at a time, in a 50 ml tube. It's the particulate carbon matter in the ink that's important, so you cannot filter the ink to make it sterile. It doesn't need to be sterile, so you're all good.**
    b. **Fill syringe with ink, attach 30g _ inch needle.**
    c. **Using forceps or your fingers, grasp the needle and bend it to make a 90° angle**
    d. **Expel any bubbles in syringe**
    e. **Insert bent needle into the yolk just under the yolk membrane a little bit away from the embryo. Avoid inserting needle into any membranes associated with the embryo**
    f. **Expel ink gently under embryo. You should be able to see the embryo nicely now**
- **Stage embryo (count somites)**
- **Using a probe, or the tip of your fine forceps gently remove the top membrane from over the embryo. Do this by scraping the tip across the surface moving from say 4 o'clock to 11 o'clock. (do not start directly over the embryo! Remove more membrane than that, so you don't poke the embryo by mistake)**

a. **The membrane is VERY difficult to see; in fact you can only see it when you remove it. So, this takes practice.**
b. **You'll be able to tell if you didn't remove the membrane because it'll be difficult to do the next step, and your pipettes will continually get plugged.**

- **Open the roof plate over the area you plan to inject using your probe**
- **Lower pipette filled with dye of choice using the micromanipulator. [I use DiI & DiD (D282&D7757 both from Probes)]**
- **Break very tip of pipette using forceps**
- **Inject at will**
- **If your pipette becomes clogged, try swabbing tip with a tiny piece of kimwipe soaked in 70% ETOH. If that doesn't work, then break the tip again. Obviously, you don't want to have to break the tip very often, or too much dye will explode out of your pipette during injections.**

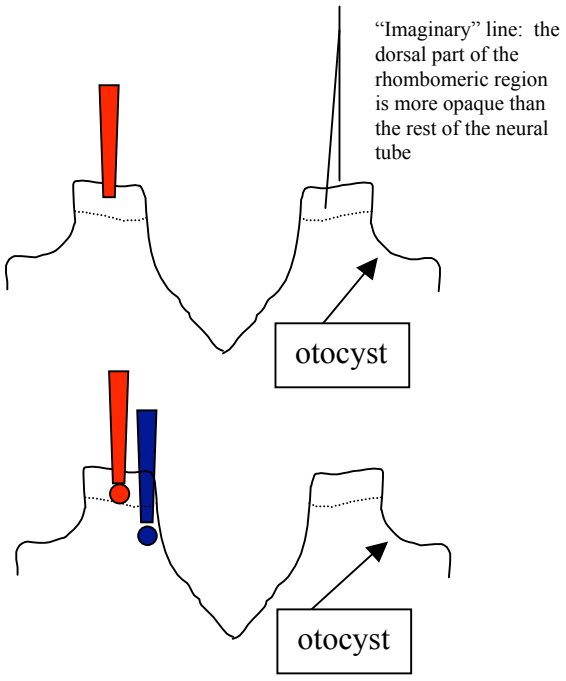
For Rhombomeric Injections:

- **Embryos should have at least 13 somites, and can be used up to about 21 somites (head will have turned at this point).**
- **Inject embryos only if their otocysts are clearly visible. This is the most salient landmark for R5/R6 injections. Grossly speaking, R5 is found as the rostral half of the otocyst, while R6 is even with the caudal half of the otocyst.**



- **Also, early embryos in particular may show a clearly delineated R4**

- **For lateral injections, aim & lower pipette at the dorsal surface of the Rhombomere. This one's easy**
- **For medial injections, aim & lower pipette at the most extreme medial edge of the dorsal boundary of the rhombomere. As you insert your pipette, it will actually enter the embryo on the slope of the neural tube. You want this injection to be separate and distinct from the lateral injection, but if you try to lower the pipette into the neural tube, your injection will turn out to be WAY too ventral. This one is very difficult.**



- **Because the embryo turns, using slightly older embryos may be beneficial for these medial injections…it's easier to get at, but as a consequence, your lateral injection is now harder. Pick your poison.**
- **It's a good idea to take a picture, and draw the 'cross-section' of your injection for fine tuning later**

Caring for your data-generating eggs

- **After injection, close the window with the strip of plastic tape.**
- **Write the experimental number and your initials on the egg, near the date.**
- **Cut 2 pieces of electrical tape just longer than the width of the plastic tape piece already on the egg**
- **Cover window completely with electrical tape, taking care to slightly stretch the tape as you're placing it to get a tight seal. Smooth out any wrinkles, and make sure the seal around the window is good and tight**
- **Place eggs in table-top incubator which should be set to 100° F and have plenty of water in all the reservoirs. Do not rotate eggs. In fact, ensure that they remain level.**

- **Make sure to check water level daily, and ensure that the cardboard egg crates do not get soaked.**
- **I get very good survival rates (85-100%), so I never peek before the date of sacrifice. It's up to you though.**
- **Dissect in PBS, postfix in 4% Para overnight. Embed in 3% Agarose, section on vibratome.**
- **As you're learning the technique, you should dissect your newly injected E2 embryos, postfix in 4% para for 2 hours-overnight, cryoprotect in 30% sucrose and section on cryostat at 10□m. Do this to ensure accurate injection placement (see the cross-section diagram above)**

Materials

Product	Cat. Number	Vendor	Address	Phone Number
Pipettes Glass filament, thin-wall 1.2mm x .9mm 4”	616000	A-M Systems	131 Business Park Loop PO Box 850 Carlsborg, WA 98324	1800.426.1306
Scotch plastic tape	191	3M,		
Scotch Super 33+ vinyl electrical tape	054007-**06132**	3M, get through University Stores		
Microfil Syringe Needle	MF28G67-5	World Precision Instruments	175 Sarasota Center Blvd Sarasota, FL 34240	941.371.1003 f941.377.5428
DiI (DiIC_{18}(3))	D-282	Molecular Probes	PO Box 22010 Eugene, OR 97402	541-465-8300
DiD (DiI C_{18}(5))	D-7757	Molecular Probes	PO Box 22010 Eugene, OR 97402	541-465-8300
India Ink, Fountain Pen Ink	46030(723)	Higgins	Bookstore	

Tools:

- **55 Dumont Fine Forceps**
- **Wax ring (~2” diameter)**
- **Medium scissors**
- **Small scissors (Roboz 5840)**
- **Sharp probe (Roboz RS6100)**
- **70% ETOH**
- **18g _ inch needles**
- **30g _ inch needles**
- **3 cc syringes**
- **Kimwipes**
- **tabletop incubator, held at 100°F with full water reservoir**
- **black plastic bag (for egg waste)**