

Drosophila Embryo Injections

for Germline Transformation

This is not intended as a comprehensive guide to fly embryo injections. Each lab has a different set-up, especially where needle pullers, micromanipulators, and dissecting scopes are concerned, which means that no detailed guide could be useful for everyone. This is just a list of things that work for me, a couple of which I made up, most of which were taught to me. Thanks in particular to Steve Small (and many others in the Levine lab ca. 1993) for teaching me how to inject.

In the Levine and Posakony labs, I have used:

- > vertical needle pullers
- > 4-inch thin-wall borosilicate glass capillaries with filament (1 mm OD/0.75 mm ID) ([WPI TW100F-4](#))
- > upright (not inverted) microscope + micromanipulator for moving needle
- > *yw* flies or *w[1118]* flies (for constructs containing *w+*)
- > 18°C cool room for lining up and injecting embryos

But other labs do it differently.

1. Supplies

- Agar plates with fruit juice (we use grape)
- Yeast paste to feed flies (I put it in a syringe)
- Glass microscope slides (I use plain, not frosted, to fit more embryos on)
- Double-sided tape (use Scotch #665; some other tapes are toxic to embryos)
- Halocarbon oil
- Embryo collection chambers that fit the collection plates, with air holes
- Needles
- Large Petri dishes with clay bars to hold needles and moistened Kimwipes
- Parafilm
- Moist plastic boxes for incubating injected embryos
- Agar plate for lining up embryos (see below)
- "Picmo" to line up embryos
- Drying box with Drierite
- 3-way or 4-way timer
- Embryo wash, "PBTx" (0.002% Triton X-100 in PBS)* in a squeeze bottle
- Small mesh basket for washing & dechorionating embryos
(can be made from the severed top 1/4 of a Falcon tube, its lid, and some plastic or metal mesh)

2. Prepare flies

I let the flies experience the collection chamber for 2-3 days before injecting, with frequent feedings. I have used 3 large chambers, or 6 small ones; this is usually overkill. You can probably put more flies in a chamber than you think. You can remove most (not all) of the males if you wish, they're just taking up space. Insert joke here. When the flies are well fed, not too old, and laying many more embryos than you can use, fewer embryos are too old to use or otherwise messed up.

3. Prepare DNA

- > Purify your transformation plasmid (I use the Qiagen midiprep kit)
- > Spin your DNA on high for a few minutes to pellet junk
- > EtOH-precipitate 20 µg plasmid with 2 µg "delta 2-3" (aka "Turbo")
- > Wash in 70% EtOH
- > Resuspend in 20 µl injection buffer
- > Max spin for a few minutes to pellet junk

4. Pull & load needles

Needle-pulling technique depends on the configuration of your needle puller. I no longer wash my needles before pulling, or break the ends. Place pulled needles in the Petri dish. Pipet up 0.4 µl* of DNA in the smallest tip you have, the push it out till it makes a drop on the end of the pipet tip. Touch the drop to the open end of the needle until it grabs on. The tip of the needle should fill by capillary action in a few minutes. Wet the Kimwipes and seal chamber with Parafilm. Can be kept at 4°C for days.

5. Break needle (can be done during first embryo collection)

- I use a 60 cc syringe and narrow tubing to push the DNA out of the needle.
- > Cut a few mm of the tubing to prevent DNA cross-contamination.**
- > Make sure you're injecting the right construct.
- > Check the needle in the dissecting scope for air bubbles near the tip. If you see one, toss the needle.
- > Carefully insert end of needle into tubing, clamp into micromanipulator.
- > Center needle tip in scope.

- > Dip needle tip into a blob of HC oil on an empty slide**, then bring it up and out of the way.
- > Place the slide at 45° angle relative to needle, to give a "beveled" break.** Locate edge of slide on scope, focus between top and bottom surfaces.
- > Carefully bring needle into focus.
- > Break tip by slowly bringing slide into contact with needle. You should be able to squeeze out some DNA. To avoid slaughtering embryos, the break should be small and subtle.
- > Leave needle tip under oil until you need it. Make a couple of nice DNA droplets in the oil to check your rate of flow.

6. Collect embryos

- > Let flies lay in fresh chambers for at least 30 min before starting 1st collection.
- > Collect embryos 60 min at room temp or 30 min at 25°C* on juice plates with a little yeast.
- > Change plates. Rinse embryos into mesh basket with squeeze bottle of PBTx. Rinse yeast off, then dry a little with paper towels.
- > Place in 100% bleach for 1 min.* Squirt bleach over embryos with Pasteur pipet.
- > Rinse for at least 1 min in alternating dH2O and PBTx, final rinse in PBTx. Dry well.

7. Desiccate

To line up embryos, I use a large Petri dish with 2x agar. I like a nice firm agar because it holds up longer against the ravages of the picmo. I add food coloring to the agar to make it easier to detect and discard over-aged embryos. I also add an antifungal (Nipagin) at the same concentration used for bacterial plates. Some people use regular-sized Petri dishes.

- > Dry the surface of the agar plate. Wet embryos stick together.
- > I use a metal poker, called a "picmo" in some circles, with a pointed end (but not sharp enough to puncture embryos) to push the embryos around. The embryos will adhere to it a little, which helps you move them. Keep it dry or they will stick too much.
- > Line up embryos - straight or staggered, but don't crowd them. I leave about 1 to 1.5 embryo-widths of space between, to avoid oxygen depletion.** I line them up from top to bottom, with their heads to the left. Identify the head by the small hairlike micropyle (sperm entryway). Also, the head is usually at the pointier end.
- > Don't use embryos with clear patches--they're too old.

> You should have glass slides with 2-sided tape lengthwise, a couple of mm from one edge. Write the name of the construct on each slide and number them so you can keep them in order. Some say you should prepare the slides the day before, so that the evil humors will have time to evaporate off the tape.**

- > Touch the slide to the embryos so that they stick a few mm from the edge of the tape, butts pointed towards the edge. I fit 50-60 embryos on a slide.
- > Place slide in drying box for 8-20 min, depending on that day's humidity. Perfect your drying time by trial and error. I do 3 slides per round.

8. Inject

- > Remove slide from drying box. Cover embryos with a minimal amount of HC oil. (Close box tightly.)
- > Raise needle. Replace empty slide with embryo slide #1.
- > Focus on rear end of embryo, then bring needle tip into focal plane.
- > If there's a drop of DNA on your needle, it can cause the embryo to leak or explode when you stick it, because it breaks the surface tension at the wound. This is especially true for underdried embryos. Move the stage quickly back and forth (<--->) to knock off the drop.

> Slowly move embryo onto needle until it punctures the membrane and pops 1/3 or 1/4 of the way in. If the embryo crumples and/or wrinkles, it's too dry. If it immediately explodes and leaks, it's underdried or your needle break is too big. Adjust the dry time on your remaining slides. You want a good conjunction between needle break size and embryo dryness. The membrane should stretch inward without crumpling, then instantly spring back when punctured.

> Once the needle pops in, slowly withdraw, applying constant pressure with the syringe. Enough DNA should be flowing that you see stuff moving inside the embryo.** Leave most of the DNA in the most posterior cytoplasm, where the pole cells will form. I also leave some in the perivitelline space, for unknown reasons.** Don't overdo it with the DNA--it risks an explosion, and probably messes with morphogen gradients and whatnot.

> Let up on the pressure as you exit the embryo, so as not to leave droplets at or near the wound. These can break the surface tension at the wound and cause embryo poppage. Also, if a little cytoplasm comes out, the embryo will probably explode later unless you "grab" it with the needle tip and move it away from the wound (this is often not possible). If lots of cytoplasm comes gushing out, give it up for dead.

> Kill embryos that are too old. If the pole cells are cellularized by the time you inject them, the DNA can't reach the nuclei. If you see clear cortical cytoplasm, a bumpy posterior surface, cell membranes, or pole cells, terminate the embryo and move on.

> Needles will clog. If this happens too often, your DNA may be unclean. In case of a clog: 1) Squeeze hard. 2) Move stage quickly back and forth (<--->) while squeezing. 3) Stab a dead embryo while squeezing. 4) Carefully re-break on edge of slide. 5) Break a new needle.

9. Incubate, scoop and mate

- > Place slide in a plastic box containing moist sponges or paper towels. Make sure that the oil doesn't run off the embryos.
- > Place box at 25°C and scoop larvae the next day, or place at 18°C and scoop in two days. Be sure to scoop the larvae before they crawl away.

> Scoop up larvae with an extra-small metal spatula, and place them in a labeled vial of fresh food. Take anything that looks well-developed, even if it hasn't hatched yet. I add a little yeast paste and score the surface of the food before adding scoopees. If survival is high, I use 2 vials for every 3 slides. Don't transfer excessive oil to the vials along with the scoopees. Your embryo survival rate will probably be terrible (<25%) at first, but this will quickly improve.

> Add a little water to the cotton plugs to keep the scoopees moist. Once they have pupated, lay the vials on their sides to discourage newly eclosed flies from falling into the food. They will eclose in 8-10 days at 25°C. Collect males and virgin females and mate them to

~3 *w*- flies of the opposite persuasion. Your transformation rate will probably be terrible (<1% of crosses) at first, but this will slowly improve.

> Now go home. You look tired.

*Or whatever you prefer

**Voodoo alert