

# Needles, and Injector for *Xenopus* Eggs

## Needles

1. The needle's number is defined as: the minimum pressure (in kPa) which is needed to blow bubbles in water (pure water, not ficoll). 50-65 kPa is a good needle for a large cell (1-16 cell embryo). For one of the 32-cell embryo cells, use like a 70-75 needle.
2. Information on injections: the total volume of an entire frog egg is about 1ul - (this is approximate) so a crude guess would be: if needle is about 65 and your injector is set at time 100 msec = you will inject about 10nl each time -my RNA is usually at 1ug/ul; and I dilute it 1:100 and inject 10nl into 1 blastomere -this should give me about 100pg of RNA with each injection (you want to keep the pg of RNA under 500pg or it can be toxic to the embryo for RNA).
3. Pull a bunch of needles, being very careful not to touch or bend the filament. Do not touch them with your hands - use gloves! Put them carefully into large petri dishes with bands of modeling clay to hold them.

## Set up the Manipulator:

1. Open the gas (on the big cylinder): back out (counter-clock-wise) the big black dial all the way; open the gray dial until 1st gauge reads the pressure in the tank. Tighten the black dial (clock-wise) until 2nd gauge reads a little more than 100. Turn on the injector machine.
2. Adjust the micromanipulator position so it's comfortable and aimed correctly.
3. Insert a needle into the holder and tighten the gasket.

## Injector (Medical Systems PLI-100)

1. Set the  $P_{\text{inject}}$  to 80 kPa (use "inject" knob)
2. Set the  $P_{\text{out}}$  to 1 (use "balance" knob)
3. Make one light shine from above, and one parallel to the surface. Fill a petri dish with clean water and put it under the scope.
4. Rinse the needle a few times by pushing and holding the "fill" button, and then pushing the "clear" button.
5. Calibrate the needle: hit "inj. time" (= 15 sec.); put a pair of forceps vertically against the bottom of the dish, and hold them there (still). With your other

hand, come along with the needle and just barely touch the tip to the metal. Pull away; you should see a bit of water enter the needle by capillary action.

6. Hit inject and twist the inject knob to lower pressure until bubbles stop; that pressure is your needle rating. If it's below 50, don't use it. If it's above 70, you can try re-breaking it by touching it to the metal again, but that almost always gives an unusably large needle.
7. Calibrate a bunch of needles between 55 and 65, and keep them labeled in a petri dish with a strip of modeling clay in it.

### To Inject:

1. Set the  $P_{\text{inject}}$  to 138-140 kPa (= 20 Psi) (use "inject" knob)
2. Put embryos in 3% Ficoll in 1X MMR (filter-sterilize and store at 14 °C).
3. Inject, by holding embryo with forceps on 1 side (closed forceps) and moving the needle in your other hand. It is **CRUCIAL** to pull out as evenly as possible, at the same angle as the puncture. Can inject with up to 50% rhodamine, to help visualize.
4. You can titrate mRNA by varying the inj. time from 240 msec to 20 msec. (for a 60 needle).
5. Keep the needle tip in water to prevent clogging.
6. wait 15 min. to 1 hr, and put them in 0.75X MMR + gentamycin (in good water, at 100  $\lambda$  per 100 ml).
7. Wait 30-45 min., and put them in 0.1X MMR + gentamycin.
8. Put them at any desired temp. and that's it! LR sidedness can be seen at st. 40 or so. Moving tadpoles can be quieted for observation by putting 6 drops of a 5% tricaine solution into a large petri dish of medium. Wash it off later though - it kills them and they become opaque.

### **Solutions you will need:**

- 1) 3% ficoll in 1X MMR: 50 ml of 10X MMR, 450 ml water, 15 g ficoll. Stir with a clean stir bar for 30 minutes or so.
- 2) 0.75X MMR: 30 ml of 10X MMR, 370 ml of water.
- 3) 2% cysteine: 600 ml water, 12 g L-cysteine, 12 NaOH pellets.