

Preparing *Xenopus* Eggs for Injection

1. Inject a female frog with gonadotropin: put 0.6-0.8 ml of "Pregnyl" (kept in the fridge with the testes) into the thinnest syringe with a short needle, and inject a frog on the side (where the "stitches" are), under the skin. Make sure not to get it into the spine, nor into muscle (if you do, the solution won't go in easy). Put the frogs in the incubators, 20-16 °C (no lower). 12-14 hours later, it is ready to squeeze.
2. Hold (very gently!) a female – she will struggle, and the eggs will come out. Try to get eggs into a dry petri dish (use the frog's butt to help spread the eggs around, into a single layer). You can let them rest for 30-45 min. (r.t. to 14 °C) and squeeze again. Don't get their jelly coat off - don't use gloves, and wash your hands before you touch them. If any frog looks sick, notify me immediately and don't squeeze it again.
3. Within 10 minutes, get some testis from the fridge, cut off a 1 mm piece, mince it with forceps and/or a micropestle in an eppendorf tube with 1 ml of 0.1X MMR in it. Put that 1 ml onto the eggs. Every 5 minutes tilt the dish, collect the 1 ml on the bottom, and put it back onto the eggs again (to make sure they all get some).
4. Wait 20 min., and flood the dish with 0.1X MMR. Fertilized eggs will turn so the pigment is on top within 20 minutes. Albinos don't have pigment so you have to wait until cleavage to figure out which got fertilized.
5. For injections, de-jelly them by adding 2% cysteine (in water) pH = 7.8 (add 2 NaOH pellets per 100 ml and check pH): pour off 0.1X MMR, add cysteine (5 min., or until they clump), rinse in 0.1X MMR. **Note:** if you cysteine too early (less than 20 minutes before 1st cleavage) you tend to de-couple the first

cleavage from the embryo's future midline.

7. Stagger the eggs into groups, by putting some at 14 and 20 °C. The start of cleavage will happen in 2-2.5 hours at 14 °C, and at 1.5-2 hours at 20 °C.

1X MMR = made from 10 X stock, pH to 7.8 and autoclaved:

10X =

58 g NaCl

1.5 g KCl

2.5 g MgSO₄

2.9 g CaCl₂

11.9 g Hepes

0.29 g EDTA

water to 1 L