

## **Xenopus laevis Protocol**

### 2 nights before (around 5pm):

- \_\_\_ Bring transportation tank, hCG, 1ml syringe, and 30 ½ gage needle down to laevis room
- \_\_\_ Prime 3 females with 50 microliters of hCG
- \_\_\_ Place 3 females in an individual tank after being primed
- \_\_\_ Cage card: Put cage card on tank with frog information completed. Extra barcode at top should be torn off and placed in plastic folder on laevis room door.

### 1 night before (around 5pm):

- \_\_\_ Fill 3 plastic tanks with water from laevis room
- \_\_\_ Put 3 primed female in one tank, and a male in a second.
- \_\_\_ Bring 3 primed females and the male up to lab
- \_\_\_ Boost females with 0.5ml hCG
- \_\_\_ Keep frogs in 16-18 °C incubator overnight

### Injection day:

- \_\_\_ Benzocaine male
- \_\_\_ Remove testes from male. Place in a dish of ice-cold 1x MR (stock kept at 4 degrees). The testes can be stored at 4°C for a week or so.
- \_\_\_ Cut a 1/4 to 1/3 of a testicle off and place in an epindorf tube filled with 0.5ml of water.
- \_\_\_ Squeeze females for eggs
- \_\_\_ Release sperm from testes with pestle
- \_\_\_ Pour sperm on eggs, mix well with pestle and wait 3 minutes
- \_\_\_ Start fertilization by flooding with water. You can put the eggs back into the incubator to slow down development
- \_\_\_ Wait 10 minutes or until you are ready to start
- ✓ \_\_\_ During this time, make 3% cysteine in 1/3 X MR PH 7.8, 2.5% ficoll in 1/3 X MR and 0.1 X MBS PH 7.8-8.0
- \_\_\_ Pull needles and rinse dishes
- \_\_\_ Once at least 10 minutes have passed, cysteine embryos for 5 minutes or until done.
- \_\_\_ Rinse 3 times with 0.1 X MBS, then 2 times with 2.5% ficoll. Leave embryos in 2.5% ficoll. They can be kept at room temperature or in 16-18 degree incubator, depending on how much time you will need for injections.
- \_\_\_ Put RNA into needle using pipette.
- \_\_\_ Break needle using tweezers, test with oil (10nl  $\cong$  13 hash marks). For injections, remove media above embryos so that surface tension keeps embryos in place.
- \_\_\_ Label dishes, after injections are done add ficoll. Rest 1 hour.
- \_\_\_ Change solution to 1/3 x MR + GENT, put embryos at 18°C or desired temp.
- \_\_\_ Change frogs to clean tank of water once they have stopped laying.