

Xenopus laevis Handling

Female Frogs:

Pre-priming injection:

-At least 2 days prior to use inject females with 100ul Pregnant Mare Serum Gonadotropin (Calbiochem #367222) 1000U/ml

Priming Injection:

-Approximately 8-12 hours prior to laying inject females with 800ul (1000U/ml) Human Chorionic Gonadotropin (Sigma CG-10).

-At room temp frogs will lay in approximately 8 hours, to slow laying they can be placed in chilled water, 12 degree water in a 16 degree incubator delays laying by 2-3 hours.

Male Frogs:

-Inject males with approximately 1ml of Tricaine (Sigma A-5040, 3-Aminobenzoic Acid Ethyl Ester) at approximately 100mg/ml (dilute in H₂O).

-Allow male to become completely anaesthetized, about 5 minutes (perform flip test, if male does not re-right self he can be sacrificed).

-Make a V shaped incision on the males belly, originally cutting only the superficial layer, then cutting through the muscles.

-Cut the descending aorta

-Locate and isolate the testes. They are attached to the fat bodies.

-Place the testes in 1X MMR + Gentamycin, store at 4°C for up to one week.

Fertilization:

-Collect eggs from females

-Pour off most of Egg Laying solution and rinse eggs once with H₂O

-Pour off as much H₂O as possible.

-Macerate a small amount of testis in 0.1X MMR using mortar/pestle.

-Drop sperm solution around dish containing eggs.

-After 5 minutes fill dish with 0.1XMMR

-Approximately 20 minutes after adding the sperm to the eggs de-jelly the eggs using 3% Cysteine pH8.