

## Observations and experiments in the living frog embryo (*Xenopus laevis*)

Excerpted and modified from: Early Development of *Xenopus laevis*: A Laboratory Manual. 2001. Sive, Grainger, and Harland. Cold Spring Harbor Laboratory Press. Many of these protocols can be found online at <http://cshprotocols.cshlp.org>.

### 1. Introduction

*Xenopus* is one of the leading vertebrate models for the study of gene function in development. The combination of lineage analysis, gene-knockout strategies, experimental manipulation of the embryo, and genomic/bioinformatic techniques, makes it ideal for studies on the molecular control of embryo patterning, morphogenesis and organogenesis. The ability to obtain embryos of all stages and the large size of the embryo (1-1.4 mm) have made detailed fate maps possible and allow the embryos to be microinjected and micromanipulated.

*Xenopus* is easy to keep, ovulates at any time of the year in response to hormone injection and develops externally and rapidly (fertilization to neurula stage takes 18 hrs). Diploid *Xenopus tropicalis* has a much shorter life cycle (4 mo) than does tetraploid *Xenopus laevis* (1-2 yrs), making it more amenable to genetics. *Xenopus laevis* is a better choice for the teaching laboratory as it is hardier and less expensive.

### 2. Where to obtain *Xenopus*

The first two sources supply only *Xenopus* and related products, thus have the most consistent quality, widest variety and best prices. Late juveniles cost less and usually yield eggs, but in smaller numbers than adults. Frogs are shipped in peat moss (or equivalent) but cannot be shipped in extreme heat or cold. Allow a minimum of 2 weeks recovery prior to experiments.

- Xenopus One [www.xenopusone.com](http://www.xenopusone.com)
- Xenopus Express [www.xenopus.com](http://www.xenopus.com)
- Carolina Biological Supply [www.carolina.com](http://www.carolina.com)
- Nasco [www.enasco.com](http://www.enasco.com)

### 3. Housing and Feeding

Females are larger than males, with a prominent cloaca. A healthy frog is placid, with moderately slimy skin and a pear shape. Do not use frogs with dry or very slimy skin, or bloated, gray, thin, or reddish frogs, as these are signs of sickness. *Xenopus* never leaves the water.

- **Containers:** Standing water tanks work for 50 or less. 4 females or 6 males per 16 liters of water, 12-20 cm deep. Plastic tanks with opaque sides approximate pond conditions. Frogs can jump up to 45 cm so tanks should be covered with a heavy lid (Plexiglas with 1/2 inch holes or stainless steel mesh). An opaque plastic pipe provides a hiding place. Water should be changed at least 3x per week.
- **Water:** Water must be dechlorinated before use, usually by exposure to air for several days in standing tubs. Chlorine and chloramines can be monitored with kits ([www.hach.com](http://www.hach.com)). A carbon filter can remove chloramines. Deaminating liquids remove chloramines and ammonia. Water can be filtered through a dirt/rust or particle filter. NaCl or Instant Ocean can be added to a final concentration of 20 mM. Rock salt (1g/liter tap water) is a less expensive alternative. If tap water quality is poor, used distilled or

reverse osmosis water supplemented with Instant Ocean to 20 mM. All of the above products can be purchased from aquarium suppliers.

- **pH:** pH 6.5 is optimal. At low pH (below 7.0) ammonia waste is present as ammonia ions, which are nontoxic. Free ammonia forms as the pH increases. A change of one pH unit (i.e. from 6.5 to 7.5) can lead to loss of the frog's protective mucus, higher susceptibility to pathogens and other stress-related conditions.
- **Light and temperature:** Frogs must be kept on a regular light/dark cycle, 12-14 hrs of light and 12-10 hours of dark. Daylight spectrum fluorescent lighting or sunlight equivalent light levels can be used. It is important to have dark areas (opaque pipes). Temperatures of 16-20°C are optimal. Above 25°C egg quality declines rapidly.
- **Food:** Adult frogs should be fed a minimum of 3 times per week, several hours before changing the water. Frogs feed well on floating food (Trout chow pellets, Purina) or frog brittle (from frog suppliers). Each adult frog will eat 5-10 pellets.
- **Seasonal Variation:** Quality of embryos varies, even in animals kept for years or bred without seeing seasonal light/dark changes. High summer temperatures (above 26°C) adversely affect egg production. Injection of frogs with 50 units PMSG (pregnant mare serum gonadotropin or hCG (human chorionic gonadotropin) a few weeks before inducing ovulation may help maintain egg production.

#### 4. Raising tadpoles and frogs

Tadpoles need 1 liter of water each; fully grown frogs need up to 4 liters each. Hundreds of tadpoles can be generated by in vitro fertilization, as described below. Unused embryos from several experiments can be pooled and reared also, but its important not to crowd dejellied embryos; they should be separated by one embryo diameter at all times. Swimming tadpole stage embryos should be transferred to a tank with good quality water containing 20 mM NaCl.

The most efficient way of generating large numbers of tadpoles is to induce natural mating. Males are injected with 300 units of hCG and females with 800 units of hCG (1000-2000 units/ml; Sigma CG10-1VL) and placed in a large tank (80 liters of water + 20 mM NaCl at a depth of ~15 cm). After 6-8 hrs, males clasp the females around the hips (amplexus) and the eggs are fertilized as they emerge from the cloaca. Its best to inject frogs in the afternoon so they will have the night to mate undisturbed. Mating stops after 12-24 hrs, after which the frogs should be removed so they don't eat their own eggs. The embryos will hatch over the next 3-4 days. Unfertilized eggs should be removed promptly. If fertilization rate is low, healthy embryos should be removed to fresh water with penicillin and streptomycin or gentamycin (0.05 mg/ml).

Tadpoles should be free swimming and ready to feed after 1 week. They can be fed daily, either a mixture of 7 parts nettle powder/ 2 parts active dry yeast/ 1 part powdered bone meal or more simply, tadpole brittle (frog suppliers). Food should be cleared within 2 hrs. Milk added dropwise until the water is slightly cloudy can be used as a weekly calcium supplement. Oxygenating the water with submerged aerators enhances growth. Water should only need to be changed weekly, with proper feeding. Tadpoles develop asynchronously with some metamorphosing within 8 weeks, and others after 4-6 months. During this time water should be no more than 30 cm deep so the froglets can reach the surface to breathe. Froglets eat trout chow pellets or frog brittle. Once ~1/3 are froglets only feed pellets or brittle as tadpoles eat broken up

food from froglets. With enough space froglets reach sexual maturity after 1 year and full size after 3 years.

### **5. Diseases, Preventions and cures**

The two most common diseases are bacterial septicemia and nematodes. Many diseases are stress-induced and occur after induced ovulation or egg collection. Females should be handled with care and isolated 24 hrs after egg collection in water supplemented with 20 mM NaCl and 5 µg/ml gentamycin. Never leave a frog in a bucket of dirty water with excess food or eggs. Other stresses include overcrowding, pH or water quality, or careless frog handling. Frogs should have moist (not excessively) slimy skin, no flaking and pigmentation is mottled but not patchy. Animals should be fat but not bloated or too thin. Skin should not be red.

- Nematode infection: symptoms are sloughing of skin, patch pigmentation, grayish and thin skin, and weight loss. Ivermectin (local aquarium suppliers) should be administered via injection into the dorsal lymph sac: 2 injections of 2 µg/g of body weight 2 weeks apart, in a volume of 100 µl.
- Red leg (bacterial septicemia): symptoms are cutaneous hemorrhage especially on flexor surfaces of thighs and foot webs, dull discoloration of skin, subcutaneous edema, and neurological disorders (trembling of limbs). Several different gram negative bacteria cause this disease. Infected individuals must be isolated and treatment is effective if administered promptly. Add oxytetracycline to the water at a final concentration of 100 µg/ml. Change the water daily and add fresh antibiotic for 7 days. Increasing salt concentration to 1 g/liter may help also.

### **6. Early development**

Shortly after fertilization, the pigmented animal cap transiently contracts, and a region of denser pigmentation becomes visible on the ventral side. The first cleavage takes about 1.25-1.5 hrs (at 22°C), and cleavages 2-12 are about 25-30 min each. Blastula stage is between 5-7 hrs post-fertilization (hpf) and gastrulation begins around 9 hpf (stage 10), when the blastopore is first visible. At these stages the animal cap is the region fated to become epidermis, the marginal zone neural and mesodermal tissue, and vegetal yolk endodermal tissues. Gastrulation ends at St 12, followed by neurulation. Embryo pictures are on the last page of this handout.

### **7. Equipment for embryo experiments**

- Variable temperature incubator or refrigerator
  - *Xenopus laevis* embryos develop between 14-23°C
  - Rate of development varies with temperature: If rate is 1x at 22°C, then the rate at 20°C is 3/4x, 16°C is 1/2x, and 14°C is 1/3x. See Appendix 1 for timing of development at 22°C.
- Dissecting microscope
  - At least 50x magnification
  - Large flat base for comfort during dissection
  - Fiber optic light source (it does not produce heat).
- Microinjector, micromanipulator, and needle pullers
  - Drummond Nanoject II microinjector
  - Marzhauser MM33 micromanipulator

[http://www.drummondsci.com/products/subpr\\_oo.html](http://www.drummondsci.com/products/subpr_oo.html)

- Sutter Instruments, Narishige for needle pullers  
[http://www.sutter.com/products/product\\_sheets/p30.html](http://www.sutter.com/products/product_sheets/p30.html)  
<http://products.narishige-group.com/group1/PC-10/injection/english.html>
- Needles
  - Glass capillaries: 30  $\mu$ l Drummond micropipette needles or World Precision Instruments glass thin wall capillaries (1mm x 10 cm)
  - For microinjection, needle tip must have tip shoulders followed by a gradual taper ~50-75  $\mu$ m in length.
  - After securing needle to injector, break tips with a forceps to a diameter of ~10  $\mu$ m. The gradual taper allows the tip to be broken repeatedly to adjust the orifice or to reopen a clogged tip.
  - The taper shouldn't be so long that it bends instead of penetrating the embryo cleanly.
- Forceps
  - Stainless steel forceps such as Dumont 5 or 5A (Fine Science tools [www.finescience.com](http://www.finescience.com)) work best for vitelline membrane removal and most micromanipulations
  - Carbon steel Dumont 5 forceps can be sharpened to a fine point if needed, using a fine stone or emery paper under the dissecting microscope, while keeping the points together. Slight bends can be introduced or straighten using the microscope stage.
- Micromanipulation tools
  - **Embryos pipettes** are used to transfer embryos from place to place. Use a diamond pencil to score a short (14.6 cm) Pasteur pipette 1.5-2 cm from the shoulders. The opening should be about 3 mm. Use a Bunsen burner flame to polish the edges so that no sharp edges remain. Alternatively, the large end of a short pipette can be used, with a bulb at the small end. Latex bulbs (1 ml) work well (i.e. VWR latex bulbs, 612-2691).
  - **Hair loops** can be easily made for steadying and moving embryos by mounting loops of human hair in beeswax at the tip of a cut off Pasteur pipette. Alternatively, pulled pipettes with rounded ends can be used. For both, heat a long (23.8 cm) Pasteur pipette below the shoulders in a small Bunsen burner flame and pull to about 15 cm
  - For the **hair loop**, heat and pull again to ~ 25 cm. Break the end after scoring with a diamond pencil. The opening should be big enough to thread the hair easily. Flame polishing of the tip smooth sharp edges. Thread a human hair longer than the pipette through the small opening until the end emerges from the larger side. Push the free end of the hair far enough into the small opening so it doesn't pop out. Tighten by pulling on the first end. Seal by scraping beeswax onto the end of another pipette and warm over flame until molten. Apply to end small opening of waxed loop.
  - For **round end pipettes**, heat end of pipette until glass softens and forms a rounded end. Experiment with size.
  - **eyebrow knives** are used for preparing animal caps or other explants; they are made similarly to hair loops. Insert the root of the hair far enough into the pipette

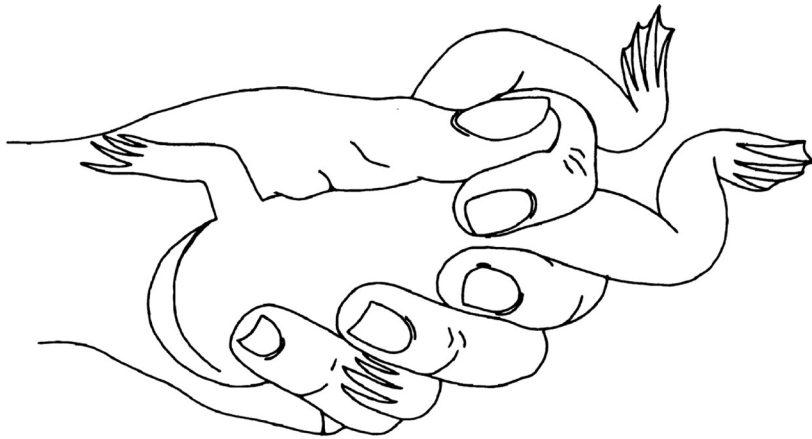
so that it is still resilient (2-5 mm) Set in place with beeswax. These knives are pushed or threaded through the tissue and flicked upward through it. They can also be used to trim isolated tissue (explants) by pressing against the solid base of the dissecting dish.

## 8. Obtaining embryos

- **Commercially:** Fertilized eggs are available from Xenopus One and Nasco. They arrive at later stages of development, depending on proximity to Michigan, Wisconsin or California.
- **Natural mating:** Males and females can be allowed to mate naturally, as described above. Eggs can be collected from the bottom with large bore pasteur pipettes (embryo pipettes). Rat cages or other containers large enough for the frogs to move about should be used. Keep frogs in 20 mM NaCl with a depth of about 6 inches. Induce ovulation by injecting females with 500-800 units of hCG. Inject males with 50 units into the dorsal lymph sac a few days before mating. This is a convenient way to obtain many different stages of embryos at once. Proven breeder pairs are available from Xenopus One.
- **Keeping track of frogs:** A frog can be induced to lay eggs repeatedly, with rest periods of 2-3 months between ovulations. Frogs can be used every 6-8 wks after natural mating. Keep accurate records of dates of ovulation, number of eggs laid, and fertilization efficiency. A frog that performs badly 3 times should not be kept. Frogs can usually be recognized by distinctive markings or coloring. It is easiest to keep batches of previously ovulated frogs together in good, medium, or poor tanks, grouped by date of last ovulation and reassessed each time.
- **In vitro fertilization**

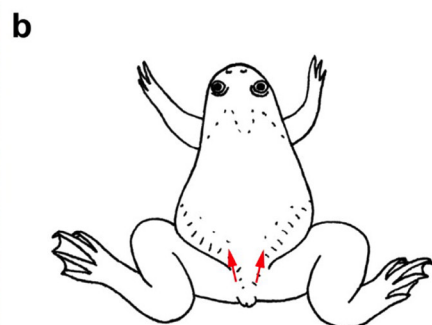
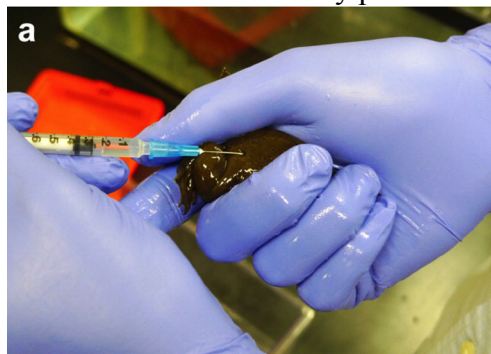
A good female lays hundreds of eggs, but since the fertilization efficiency varies widely, ovulation should be induced in more than female (2-3). The testes from one male contain enough sperm to fertilize several thousand eggs.

  - **Handling Xenopus adults:** Handle frogs with clean, soap-free hands or smooth latex gloves. Powdered or textured gloves abrade the frog's skin.
    1. Pick up the frog by placing one hand across its back with a forefinger between the animal's hindlegs and wrapping the rest of the hand around the animal's middle (see picture below).
    2. Use the other hand to cover the frogs eyes, to calm and physically restrain it. Escaped frogs dehydrate quickly and die with hours, so must be captured quickly and rinsed well in distilled water.



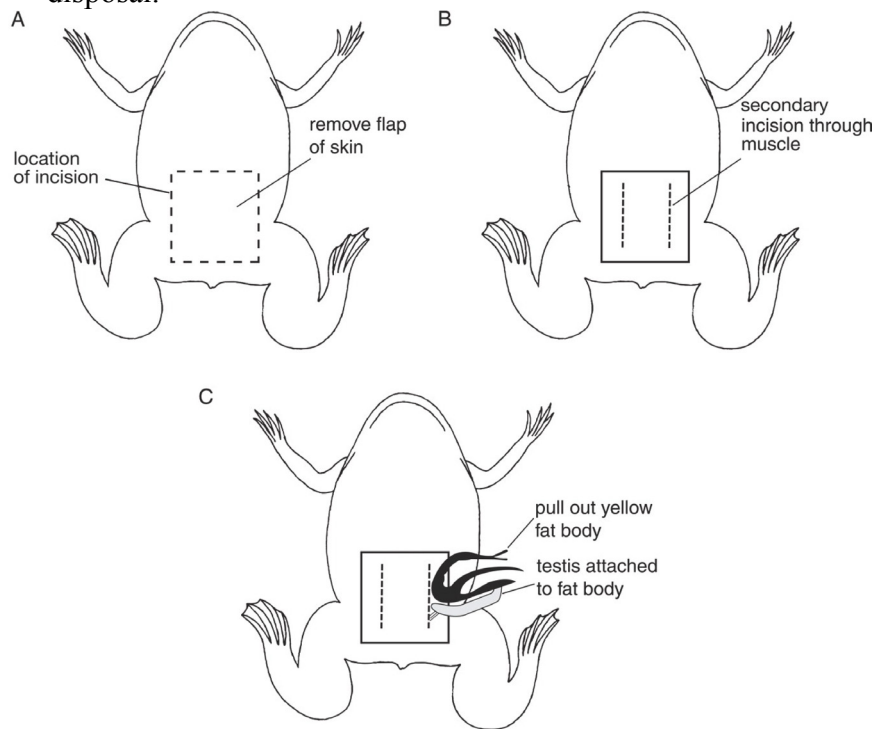
- **Inducing ovulation:** Ovulation is induced by injection of hCG (available from Sigma) into the dorsal lymph sac of a female frog. Newly purchased frogs and those that have not ovulated for 6 months should be primed before induction of ovulation. 50 units of hCG should be injected into the dorsal lymph sac at least 5 days before ovulation. Optimal egg quality is obtained 1-2 weeks after priming. For induction of ovulation 500-800 units of hCG (depending on frog size) should be injected. Frogs kept at room temperature (23°C) begin laying eggs 9-10 hrs after induction of ovulation. Frogs kept at 15°C begin egg laying ~ 14 hours after injection.

1. Wrap the frog firmly in wet paper towels, covering the eyes, but leaving the hind legs and lower abdomen exposed (step can be omitted with experience).
2. Place frog belly-down on a clean smooth surface.
3. Place the middle and index fingers over the frog's thighs so that pressure can be exerted on its thighs to prevent movement.
4. Use a fine (26 gauge) needle attached to a 1-ml syringe and place the needle posteriorly, at the level of the hindlimb near the lateral line sense organs. Penetrate the skin with a firm push holding the syringe almost parallel to the back. Pushing the needle straight down will penetrate the muscle beneath and cause bleeding.
5. If skin is penetrated outside the lateral line, slip the needle laterally toward the dorsal midline, across the lateral line "stitch" marks to the dorsal lymph sac. If penetration was inside the lateral line, push the needle gently directly down into the lymph sac.
6. Penetrate the wall of the sac (resistance will be felt) and inject the liquid. Wait 5 seconds and slowly pull out the needle.



○ **Isolating the testes**

1. Sacrifice a male frog by submerging in tricaine at room temperature for 30 min to 1 hour. When laid on its back the frog should be completely limp and the heart should not be beating. Prepare fresh solutions of tricaine and store at 4°C.
2. Place the frog belly up on clean wet paper towels. Use a tissue or sharp forceps to pick up the loose skin on the belly and make a small cut with scissors. Cut a large flap of skin open to expose the lower abdomen.
3. Lift the abdominal muscles with forceps and cut to expose the viscera by making a slit on either side of the dorsal midline.
4. Use blunt forceps to push aside the liver and pull out the yellowish fat body and thus the testes. The testes lie at the base of the fat bodies, are whitish, 1 cm long and covered with capillaries.
5. Use scissors or forceps to free each testis from the fat body and surrounding connective tissue
6. Place the isolated testes in 80% calf serum, 20% 1x MMR with antibiotic (0.05 mg/ml gentamycin) and store at 4°C. Under these conditions, testes can be kept for at least 48 hours, after which sperm viability drops.
7. Wrap the frog carcass and freeze at -20°C for at least 24 hrs before disposal.



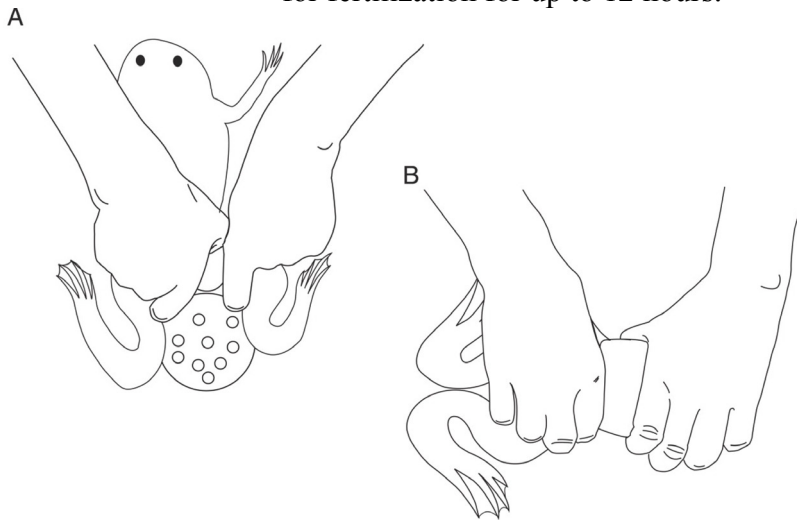
○ **Collecting eggs**

Keep females in 20mM NaCl. Supplemented with 5 µg/ml gentamycin during egg laying and 12 hours after. Keep in isolation in clean water for 24 hours after egg collection to monitor for signs of illness. High quality eggs are laid singly, not in clumps or strings. They should be round and firm. White eggs are poor quality. Pigmented eggs should have a clearly defined animal hemisphere and be of uniform size.

1. Eggs collect in a sac near the cloaca; simultaneous lateral and vertical pressure should expel them. Hold the frog with two hands (A) and gently

but firmly massage the belly with one thumb over a clean Petri dish (80 mm) containing 0.1 X MMR. She should begin to lay within 1 minute. If not, repeat massage with increased pressure. Egg collection should not last more than 2-3 minutes total. Make collections every hour for the first 2-3 hours and then less frequently as the day progresses. A maximum of 4-6 collections can be expected from 1 frog in 1 day.

2. An alternate method (B) is to place the frog belly down on clean foil or plastic wrap. Hold a hand over her, making sure her eyes are covered, and stroke firmly down either side with fingers of the other hand. Eggs are collected dry and washed off into a Petri dish containing 0.1x MMR.
3. Do not rub the back of the frog! There is little fat here and it will bruise her.
4. Eggs can also be collected from females maintained in 1x MMR after they have been laid in the container. Eggs must be removed soon after laying for optimal fertilization. (15 minutes or less). Eggs can remain competent for fertilization for up to 12 hours.



#### ○ **In vitro fertilization**

1. Eggs that will be fertilized immediately can be collected into 0.1x MMR. Otherwise maintain in 1x MMR. Just prior to fertilization remove all buffer from the eggs.
2. Use forceps (cleaned with 70% ethanol) to tease a piece of testis apart and rub the tissue over the eggs, touching every egg.
3. Alternatively, crush part or all of the testis in 1x MMR and mix this (~1/20) with the eggs.
4. After contact with the sperm, flood eggs with 0.1x MMR.
5. A few minutes after fertilization the animal hemisphere contracts to less than 1/2 of the egg and the egg becomes rigid. About 20 min after fertilization the eggs rotate within the vitelline membrane so the animal hemisphere faces up. 80-100% fertilization rates occur with good quality eggs.

## 9. Preparing embryos for manipulation

### • **Dejelling the embryos**

1. Remove buffer and swirl gently in 1 x MMR with 2% (w/v) cysteine at pH 8.0.



2. Gently swirl the eggs for 2-4 minutes until the jelly membranes are visible in the solution and the eggs start to pack together, with no spaces between.
3. Dejellifying is usually complete within 4 minutes but varies from frog to frog.
4. When embryos begin to pack, decant the cysteine and rinse at least 5 times in 0.1 x MMR over a period of about 10 minutes. Rinse them in a clean beaker.
5. Place rinsed embryos in a clean dish. Keep at a density of 100 per 80-mm Petri dish in 0.1X MMR and remove dead and unfertilized embryos promptly

- **Removing the vitelline membrane**

The vitelline membrane must be removed for microdissection, but not for microinjections.

1. Place the dejellied embryos in a clean Petri dish coated with 1% agarose in water, containing 0.1x MMR.
2. Use a pair of sharp forceps (with points bent toward each other like pinchers) to take hold of the vitelline membrane.
3. Use a second pair of forceps (slightly blunt tips) to steady the hold on the embryo
4. Tear the vitelline membrane with the sharp forceps.
5. Store the naked embryo in 0.1x MMR.

## 10. Embryo perturbations

Xenopus axis formation can be perturbed to various developmental stages to give rise to a range of altered (dorsalized or ventralized) phenotypes. The Dorsoanterior Index (DAI, Kao and Elinson, Dev Biol 127:64-77,1988) can be used to classify the phenotype, with 0 assigned to embryos lacking dorsoanterior structures and 10 assigned to embryos with extreme dorsoanterior structures. Untreated embryos should be set aside for controls. For our purposes, a listing of techniques and their result will be presented. Details can be found in the Xenopus lab manual.

- **Axis perturbation by UV treatment:** Treatment with ultraviolet light during the first cell cycle prevents rotation of the cortical cytoplasm that establishes dorsoventral polarity. The resulting ventralized embryos do not develop a dorsal blastopore lip in early gastrula stage. Instead they develop a circumferential blastopore lip at St 11, when the ventral blastopore lip usually develops. Embryos can be sorted at St 10.5, when those lacking the dorsal lip will be the most severely ventralized.
  1. Embryos should be dejellied right after fertilization and placed in 50-ml orange cap tubes (Corning) containing 45-ml 0.1x MMR, sealed with saran wrap and a rubber band.
  2. Place inverted tube on a UVGL-25 lamp (VWR) and irradiate different batches of embryos for 30, 60, or 90 seconds with short wave (254 nm) UV light
  3. Sort out dead embryos before blastula stage (~50% mortality expected).
  4. Transfer embryos to agarose-coated dishes and incubate below 20°C to improve survival.
  5. Identify axis deficit embryos at St 10-10.5. Score using the DAI during neurulation (St 25-35) or tadpole stages (about St 40).
- **Axis perturbation by Lithium chloride treatment:** Treatment with lithium chloride results in dorsalization, acting via inhibition of glycogen synthase kinase-3 $\beta$ , which allows *wnt* activation for dorsal axis formation.

1. Dejelley eggs before the 32-cell stage.
2. Transfer embryos to a Petri dish containing 0.3 M LiCl in 0.1X MMR. Swirl to equalize concentration.
3. Incubate embryos at 18-20°C for 10 minutes.
4. Rinse well and transfer embryos to a clean Petri dish with 0.1X MMR.
5. Allow embryos to develop at 18-20°C. Score perturbations at early gastrula, when the dorsal blastula lip forms synchronously around the embryo. By tailbud or hatching, dorsalized embryos have exaggerated heads (DAI>5), which can be radially symmetrical with bands of eye pigment and cement gland and a cylindrical heart in the most extreme causes (DAI 10).

## 11. Microinjection

Many experiments use microinjection and the procedure can be performed on oocytes (not covered here) or embryos. Embryos can be microinjected with mRNA or DNA constructs at the single cell stage or later. RNA injection allows uniform expression over a large region of the embryo, with translation starting immediately. Promoter-driven DNA constructs are transcribed only after the midblastula transition (5-7 hrs), when global transcription begins. DNA expression is mosaic, with many cells not producing any exogenous protein. A maximum of 5 ng of mRNA and 100 pg of DNA should be injected. Volumes for 1-4 cell embryos should be kept to 10 nl or below. The jelly coat must be removed prior to injections, usually 30 minutes after fertilization. Dejelled embryos should be transferred to injection dishes containing 2-5% Ficoll 400 in 0.1x MMR. This collapses the vitelline space, reducing pressure on the embryo and preventing cytoplasmic leakage.

- **Injection checklist**

- 2% cysteine (w/v) in 0.1x MMR, pH 8.0. Prepare fresh day of injection.
- 70% ethanol (to sterilize instruments)
- 3% Ficoll in 0.1x MMR
- Hairloop or round end pipette
- Microinjector
- 0.1x or 1/3x MMR
- 1x MMR
- mRNA or DNA (or quantum dots) for injection. Prepare fresh dilutions on day of injection.
- Injection needles
- Injection dishes

- **Preparation of injection dishes**

- Injection dishes are small Petri dishes to which a nylon mesh is fixed to hold embryos in place.
- For mesh-lined dishes, cut a sheet of 800 µm Nitex screen (Tetko) into circles that fit snugly into Petri dishes.
- Fix the mesh in place by melting the bottom of the plastic dish with 5 drops methylene chloride or chloroform (use a silicone rubber cork to hold the grid flat while plate sets).
- Rinse dishes thoroughly with 95% ethanol and then distilled water after solvent evaporates.

- Alternatively, use glass Petri dishes lined with modeling clay, into which small wells are depressed.
- **Calibration of injection volume**
  - For a pressure injector, backfill the needle with an automatic pipettor fitted with a long narrow tip and mount on the injector.
  - For the Drummond Nanoject, the needle is back filled with mineral oil using a 2” 30 gauge stainless steel needle (Drummond) attached to a 1 ml syringe, mounted on the injector and then front filled with injection solution (placed first on a piece of parafilm on the dissecting scope stage).
  - Break needle tip to produce an orifice of ~ 10 μm (under dissecting scope).
  - Place a small drop of paraffin or mineral oil on a microscope slide (or parafilm) and place on stage of dissecting microscope.
  - Calibrate the eyepiece micrometer for appropriate magnification and perform a trail injection into the drop of oil. The injected liquid forms a sphere within the oil droplet.
  - Measure the diameter of the sphere using the eyepiece micrometer and calculate the injected volume ( $v = 4/3\pi r^3$ , where  $v$  is volume and  $r$  the radius of the sphere).
- **Injection of embryos**
  - Embryos can be injected from the 1-cell to 32-cell stage, but the injection volume should be below 10 nl.
  - Fill the injection dish with 3% Ficoll in 0.1X MMR.
  - Line up the embryos to be injected, choosing either obviously fertilized embryos (firm and free floating) for the 1-cell stage or embryos at the appropriate stage. We most often inject into 1-cell of the 2-cell embryo.
  - For best diffusion of the mRNA or quantum dots in our case, we inject into the animal cap, where diffusion occurs more rapidly.
  - The needle should pierce the embryo easily, after an initial resistance; keep the needle in place for 1-2 seconds after injection.
  - Once all embryos have been injected, transfer to a dish filled with 3% Ficoll in 0.1x MMR. Transfer out of the Ficoll by the MBT if later development will be assessed.

## 12. Fate mapping and lineage labeling

Please see the Xenopus manual for detailed protocols.

## 13. Microdissection

The majority of the microdissection protocols found in the Xenopus manual can be found online at <http://cshprotocols.cshlp.org>.

## 14. Solutions

### MMR (Malters Modified Ringers) 1 liter

Buffer for frog testes (1X) and embryo culture (0.1X).

<u>1X</u>	<u>10X</u>	<u>g for 10X</u>	<u>MW</u>
0.1M NaCl	1M NaCl	58.44	58.44
2mM KCl	20mM (0.02M)	1.49	74.56

1mM MgSO <sub>4</sub>	10mM (0.01M)	1.20	120.37
2mM CaCl <sub>2</sub>	20mM (0.02M)	2.94	147.02
5mM Hepes	50mM .05M)	11.915	238.3

1. In a 1-liter beaker on stirrer with stir weight, dissolve chemicals in ddH<sub>2</sub>O.
2. pH to 7.8. Use HCl to lower pH. Use NaOH to raise pH.
3. Bring solution to 1 liter in a graduated cylinder.
4. Transfer to 1 liter bottle and autoclave.

### **Tricaine methanesulfonate-S (MS222)**

Used for anesthetizing female frogs for oocyte removal or for euthanizing male frogs. Tricaine is stored at -20°C. 1g/liter is used to anesthetize female frogs (~ 10 minutes) or to euthanize male frogs (30-60 minutes at room temperature). Alternatively, male frogs can be injected with 0.5 ml of 20 g/liter.

1. In an appropriate sized beaker on stirrer with stir weight, dissolve tricaine in ddH<sub>2</sub>O.
2. Add a little Hepes Buffer for easier pHing.
3. pH to 7.0 with NaOH Store at 4°C, bring to room temperature for use.

### **2% Cysteine**

Used to dejelly eggs. Make fresh for each use.

1. In a 100 ml beaker on stirrer with stir weight, dissolve 2g Cysteine in 100ml 0.1x MMR.
2. pH to 7.8-8.0 with NaOH (starting pH 0.9)

### **3% Ficoll**

In a 100ml beaker on stirrer with stir weight, dissolve 3g Ficoll in 100ml 0.1X MMR.

### **Reference books/videos on *Xenopus laevis***

- **Early Development of *Xenopus laevis*: A Laboratory Manual. 2001. Sive, Grainger, and Harland. Cold Spring Harbor Laboratory Press. <http://www.cshlpress.com>**  
The majority of information on this handout was excerpted from this exceptional book, which was produced from and for the Cold Spring Harbor *Xenopus* course. It is available on request through the above website.
- **Manipulating the Early Embryo of *Xenopus laevis*: A Video Guide. 1999. Grainger and Sive, Eds. Cold Spring Harbor Laboratory Press. <http://www.cshlpress.com>**  
This video supplements the manual above is currently available only on video.
- *Xenopus laevis*: Practical Uses in Cell and Molecular Biology, Methods in Cell Biology, Vol. 36. 1991. Kay and Peng, Eds. Academic Press.
- **Normal Table of *Xenopus laevis* (Daudin). 1994. Nieuwkoop and Faber, Eds. Garland Publishing, Inc.**
- Johnson and Volpe's Patterns & Experiments in Developmental Biology, 3<sup>rd</sup> Ed. 2001. Leland Johnson. McGraw Hill.

## Websites

- <http://cshprotocols.cshlp.org>
- <http://www.xenbase.org/common/>
- <http://faculty.virginia.edu/shook/ShowMovies/>
- <http://worms.zoology.wisc.edu/frogs/welcome.html>
- <http://www.nih.gov/science/models/xenopus/>
- [www.swarthmore.edu/NatSci/sgilber1/DB\\_lab/Frog/JA\\_Spenillo/justin2.html](http://www.swarthmore.edu/NatSci/sgilber1/DB_lab/Frog/JA_Spenillo/justin2.html)
- <http://embryology.med.unsw.edu.au/OtherEmb/Frog.htm>

## Abbreviated Table of *Xenopus laevis* embryonic development

Stage	Hours at 22°C	Description
1	0	1 cell
2	1 1/2	2 cells
3	2	4 cells
4	2 1/4	8 cells
5	2 3/4	16 cells
6	3	32 cells
6 1/2	3 1/2	about 48 cells
7	4	large cell blastula; tangential cleavage
8	5	medium cell blastula
9	7	fine cell blastula
10	9	initial gastrula; pigment concentration at blastopore
10 1/4	10	early gastrula; dorsal blastopore groove
10 1/2	11	crescent-shaped blastopore
11	11 3/4	horseshoe-shaped blastopore
12	13 1/4	medium yolk plug stage
13	14 3/4	slit-blastopore stage
14	16 1/4	neural plate stage
15	17 1/2	early neural fold stage
16	18 1/4	mid neural fold stage
18	19 3/4	neural groove stage
19	20 3/4	initial neural tube stage
20	21 3/4	neural folds fused; suture still present
24	26 1/4	initial motor reactions to external stimulation
25	27 1/2	beginning of fin formation
29/30	35	tail bud distinct
33/4	44 1/2	melanophores appearing dorsally on head
35/6	50	melanophores appearing on back
40	66	mouth broken through; outlines of proctodeum and tail myotomes form 90° angle

