In Vitro Fertilization of Xenopus Laevis Frog Eggs

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We purchase our *Xenopus laevis* from Nasco (http://www.enasco.com/page/xen_price), and have been very satisfied with their product.

-Female frogs should be primed with human chorionic gonadotropin (HCG) the night before you need eggs (Sigma Cat # CG10-10VL. Each vial contains 10,000 U of HCG; resuspend this in 10 ml H_2O, giving a final concentration of 1,000 U/ml). Inject 500 units of the resuspended HCG in the female frog's lymph sac approximately twelve hours before eggs are desired.

-Keep the primed female housed in charcoal filtered water overnight. Dump the water in the morning, and have the primed female lay the eggs in a 1X High Salt Barth's solution made with charcoal filtered water.

-Excise the testes from a male frog, and keep on ice.

-Collect the eggs using a pipetman outfitted with a 25 ml pipet that has had a little bit of its tip sawed off with a hacksaw, and then smoothed out with some emory cloth. Always have the pipetman set to the slow setting while collecting the eggs. **As much as possible try to avoid any perturbations of the eggs at all points during the process of fertilization**. Pipet the eggs into a 100 mm glass petri dish, trying to limit as much as possible the amount of buffer, covering about one third the surface of the plate. Carefully pipet off any extraneous buffer.

-Take about one half of one male testis and place in a 1.5 ml eppendorf tube. Add approximately 500 µl of 1X Steinberg's solution and chop up the testis thoroughly with surgical scissors until the contents of the tube are semi-homogeneous. Add additional Steinberg's solution to the top of the eppendorf tube. Take up some of this suspension in a Pasteur pipet, and place ten or so drops on the eggs collected in the petri dish. Disperse the testis suspension throughout the eggs by gently swirling the remaining intact testis through the eggs while being held with a # 3 Dumont forceps. (Each subsequent fertilization should use a fresh one half of a testis which has been pulverized in 1X Steinberg's solution).

- Incubate at Rm temp 5 min.
- Gently pour 0.1 Barth's solution over the eggs, trying to disrupt them as little as possible. Incubate 15 min Rm temp.
- Gently decant off the 0.1X Barth's solution.
- Gently add cysteine solution, trying to disrupt the eggs as little as possible. Incubate 5 min Rm temp.
- Aspirate off the cysteine.
- Gently add more cysteine.
- Incubate 2 min Rm temp.
- Aspirate off cysteine.
- Gently wash 6 X with 0.1X Barth's, aspirating off buffer each time to remove cysteine.
- Add 0.1X Barth's solution and incubate eggs. The first doubling should occur approximately one hour and twenty minutes after the eggs are first covered with 0.1X Barth's solution.

Solutions:

Note: All solutions should be made using nanopure water.

10X High Salt Barth's Solution: Fill a 4L beaker to 3L with nanopure water and add following:

Compound	Amount	Final Conc
NaCl	256 g	1.095 M
KCl	3 g	10 mM
$NaHCO_3$	8 g	24 mM
$MgSO_4.7H_2O$	8 g	8 mM
Hepes	95.2 g	100 mM

Add the following dropwise after dissolving each in 25 ml nanopure water:

Compound	Amount	Final Conc
$Ca(NO_3)2.4H_2O$	3.2 g	3.4 mM
$CaCl_2.2H_20$	2.4 g	4 mM

pH to 7.7 with HCl
Bring to 4L with nanopure water
Important: When making a 1X dilution always use nanopure water
When making a 1X dilution add 1 ml of 5 % ampicillin per liter

10X Barth's Solution: Fill 500 ml beaker to 350 ml with nanopure water and add following

Compound	Amount	Final Conc
NaCl	26 g	889 mM
KCl	0.38 g	10 mM
$NaHCO_3$	1 g	24 mM
Hepes	11.9 g	100 mM

Add the following dropwise after dissolving each in 25 ml nanopure water:

Compound	Amount	Final Conc
$MgSO_4.7H_2O$	1 g	8 mM
$Ca(NO_3)_2.4H_2O$	0.4 g	3.3 mM
$CaCl_2.2H_2O$	0.3 g	4.1 mM

Bring to pH 7.6 with NaOH
Bring to 500 ml with nanopure H2O
When making the 1X and 0.1X dilutions add 1 ml of 5 % ampicillin per liter

10X Steinberg's Solution: Fill 1 liter beaker to 800 ml with nanopure water

Compound	Amount	Final Conc
NaCl	34 g	581 mM
KCl	0.5 g	6.7 mM
$Ca(NO_3)_2.4H_2O$	0.8 g	3.3. mM
$MgSO_4.7H_2O$	2 g	8 mM
Kanamycin	0.1 g	0.01 %
Tris Base	6 g	50 mM

pH to 7.35-7.45 with HCl
adjust volume to 1 liter with nanopure H2O

2% Cysteine Solution: Fill a 1L beaker to 800mL with 0.1X Barth's
Add 20g L-Cysteine Hydrochloride Monohydrate (Sigma cat # C7880-500G)
pH to 7.8 with NaOH
Bring to 1 liter with 0.1X Barth's

5% Ampicillin:
Dissolve 5 grams Ampicillin in 100 ml nanopure H_2O (Vendor: Sigma-Aldrich, Catalog #: A9518)
Filter sterilize, make 1 ml aliquots in 1.5 ml eppendorf tubes, and store at -20°C.