Embryo Microinjection

Ryan Kerney November 2004

All chemicals should be made in autoclaved animal safe glassware.

2% Cysteine

40 mls of 0.1X MMR
1 g of L-cysteine (sigma C-7880)
2 small chips of NaOH
pH to 8.5 with animal safe probe or in a separate container

4% ficoll in 1/3 x MMR

add ficoll slowly while stirring

10X Marc's Modified Ringers (MMR)

1M NaCl
20 mM KCl
10 mM $MgSO_4$
20 mM $CaCl_2$
50 mM HEPES (pH 7.8)
1 mM EDTA

Modified Barth's Saline with HEPES buffer (MBSH)

Prepare separately 0.1 M $CaCl_2$ 11.1g / liter
Autoclave and store in aliquots at –20C and –4C
10x MBS Salts

880 mM NaCl
10 mM KCl
10mM $MgSO_4$
50 mM HEPES (pH 7.8)
25 mM $NaHCO_3$
Adjust to final pH of 7.8 with NaOH
Mix 100 mls of 10X MBS Salts with 7 mls of $CaCl_2$ and adjust volume to 1 liter with distilled water

Chorionic Gondaotropin (Sigma CG-10)

Dilute lyophilized stock to 1000 units per ml by adding 10 mls of SUPW to 10,000 unit stock

10% MS-222 (Sigma A-5040)

pH to 7.5

Priming female Frogs

* Choose two female frogs which have bulges around hips and enlarged cloacas.
    *Pre-prime with 25ul (25 units) of HCG, into the back of the female frog just medial to the lateral line, three days before injections
    * Prime with 300ul (300 units) of HCG on the opposite side.
    * Make sure the needle is not pointed towards the spinal chord.

Collection of Testes

Euthanize a male frog in a shallow bath of 10% MS-222 for 30 minutes.

Testes are "universal white" and surrounded by fat in the lower abdominal cavity

Try to sever all surrounding blood vessels as closely to the testes as possible

Store Testes in cold MBSH

Pulling Needles

Use World Precision Instrument Glass replacement Pipettes (7.0 nl)

Set Pul-1 to a delay of 4 and heat of 10

Tighten needle firmly into holders so that the center is just above heating element

Calibration of Injection Volumes

Use no more than 15 nl for a one cell embryo and 10 nl for a two cell embryo

mRNA	5 ng
Plasmid DNA	100 pg
Morpholinos	5-10 ng (5-10 of 1 mM C1)

Gives appx 1-10 uM final concentration in 1 ul oocyte

Focus on the end of the needle and quickly measure the droplet size after injecting a droplet into the air. Alternately inject a small droplet into a drop of paraffin oil. (Mineral oil will not work since it is less dense than water!)

Set the scope to the 12X magnification with 10X oculars.
1 small graduation equals 80 um.

Small Graduations	**Diameter**	**Volume $4/3\pi r^3$ (nl)**
1	**80 um**	**0.27 nl**
2	**160 um**	**2.14 nl**
3	**240 um**	**7.24 nl**
3.5	**280 um**	**11.49 nl**
4	**320 um**	**17.15 nl**
5	**400 um**	**33.51 nl**
6	480 um	57.91 nl

Injections

Calibrate the needle and place embryos into ficoll solution over Nitex mesh.

Clip the needle with a pair of foreceps so that its tip is appx 80 um.

Orient embryos so that the animal pole is up by gently rocking the tray.

Reduce the level of ficoll so that the embryos are just barely covered.

Inject DNA near the animal pole and RNA and morpholinos near the center of the equator.

Hold the needle for 1 second before slowly withdrawing it and moving to the next embryo.

Transfer the embryos to a new tray of 1/3X MMR without ficoll, and leave them at 18C overnight.

Score the injections the next day and replace the 1/3X MMR with 0.1X MMR