XENOPUS OOCYTE MICROINJECTION

Materials:

ORII SOLUTION pH = 7.6

	MW	mM	g/liter	g/500 ml
NaCl	58.44	82.5	4.822	2.411
KCl	74.55	2.0	0.150	0.075
$MgCl_2.6H_2O$	203.3	1.0	0.240	0.102
HEPES	238.3	5.0	1.20	0.60

MODIFIED BARTH'S SALINE

	mM	To prepare:
NaCl	88.0	
KCl	1.0	919 ml dH_2O
$NaHCO_3$	2.4	40 ml high salt solution
HEPES	15.0	40 ml divalent cation stock
$NaNO_3$	0.3	1 ml antibiotic stock (10 mg/ml gentamycin)
$CaCl_2 \cdot 2H_2O$	0.71	
$MgSO_4$	0.82	

DIVALENT CATION STOCK

	MW	mM	g/liter	g/200 ml
$NaNO_3$	84.99	8.05	0.687	0.137
$CaCl_2 \cdot 2H_2O$	147.02	17.78	2.62	0.523
$MgSO_4$	120.37	21.70	2.612	0.523

Store 40 ml aliquots at -20°C

Procedure:

A. MAINTENANCE OF FROGS

Adult Xenopus frogs can be purchased from Nasco, Inc. Phone (414) 563-2446
901 Janesville Ave., Fort Atkinson, WI 53538

The frogs are housed in 16x11x6 inch plastic tanks containing 3-4 inches of dH_2O and kept at 19-20°C. The lids should contain about 20 holes (approximately 1 cm in diameter). The frogs are kept in a light/dark cycle, fed 2x a week with Frog Brittle (Nasco), and the water should be changed once a week.

B. SURGERY

1. Prior to surgery, the frog is anesthetized in 1 liter of water containing 0.1% ethyl m-aminobenzoate (MW 222) for 20-30 minutes--or until the frog does not move when pinched. The frog is then laid down on a paper towel, tummy side up.
2. An incision of about 1 cm is made first by cutting through the skin, then through the muscle.
3. Part of the ovary is carefully pulled out, tied on the back, cut off, and placed in MBS.
4. The incision is sewn together through both layers (first muscle, then skin) with two stitches.
5. For recovery, the frog is covered with wet cheese cloth (except her head) at a slightly elevated position so that only her body is in the water, not her nose, to prevent drowning. After recovery she is returned to her tank filled with water.

C. OOCYTES

1. The membrane is broken by teasing it with tweezers under a microscope.
2. The oocytes are then placed in 8 ml of ORII solution containing 2 mg/ml collagenase (Sigma).
3. After shaking for 1.5 hour, the oocytes are washed 2-3x with ORII solution and again placed in 8 mls of fresh 2 mg/ml collagenase and shaken for another 1.5 hrs.
4. Wash 2-3x with MBS and transfer to petri dishes which contain MBS at 19°C.

D. INJECTION

1. Micropipettes of 150-180 microns in size are prepared using a needle puller and a microforge.
2. The micropipette is filled with parafilm oil then connected to the apparatus via flexible polyethylene tubing. The DNA solution (3-6 µl) is then loaded avoiding air suction. Oocytes are arranged animal-pole (black side) upward on top of a small plastic grid in a petri dish containing MBS. This should be done carefully using a pasteur pipet as the oocytes are fragile and rupture easily.

3. Insert the micropipette into the center of the pigmented region (the animal pole) and inject the DNA (10-15 µl).
4. After injection, incubate the dishes at 19°C for the amount of time desired.

E. PREPARTION OF OOCYTE EXTRACT

1. Homogenize 10-30 oocytes in 100 µl 250 mM Tris pH 8.0 by pipetting up and down.
2. Spin for 15 minutes at 15,000 rpm.
3. Remove the supernatant (avoid sucking up the utmost yucky stuff).
4. Spin again for 15 minutes at 15,000 rpm.
5. Use the supernatant for assays.