INTERPHASE EXTRACTS, WALTER (MSS)

Initials SR

Reference Walter lab modification, derived from Blow.

Recipes used in protocol	Amount Used
EB, 1x	200 ml
EDBS	50 ml
Energy mix, 35x	
MMR, 1x	4 liter

Protocol

This is a modification of the original Murray protocol. This seems to be much more stable at -80, perhaps due to the reduced amount of mitochondria?

1) Induce egg laying in desired number of frogs by injection with HCG. Allow frogs to lay out overnight in 1 x MMR

2) Collect eggs in a beaker and wash 2X with MMR.

3) Dejelly eggs in 2% cysteine in 1xMMR. The pH must be adjusted to 7.8-8.0!

4) Wash eggs 3x in 1xMMR, once in 0.2x MMR.

5) Activate eggs in ionophore (A23187; 20 mg/ml is 10,000X) prepared in 0.2xMMR. Do this by pouring off as much MMR as possible, then adding MMR containing ionophore to the eggs. Incubate for 3 minutes. You should see the eggs starting to activate.

6) Immediately wash eggs 3x in 0.2xMMR. The eggs will continue to activate.

7) Wash the eggs 4x in EB at 4C (all remaining steps at 4C). Pour eggs into Falcon 2059 tubes. Remove as much buffer as possible. Let settle and top off with eggs. Repeat if necessary.

8) Packing spin: spin in 2059 tubes either 1200 rpm 1' in clinical centrifuge in lab, or spin 1min 1000rpm in JS13.1 rotor. Remove all buffer from the top of the tube.

9) Crush the eggs by spinning 10', 10,000 rpm in swinging bucket rotor (JS13.1).

9) Remove cytoplasm to 50 ml conical tube. Do this with short Pasteur pipette. Push aside lipid layer (yellow) and gently remove tan layer in middle of tube. Don't worry about bringing along some lipid at this point.

10) Add:
- EDBS to 15% of the total cytoplasmic volume.
- Cytochalasin B to 10 µg/ml (from 10 mg/ml stock in DMSO)
- Cyclohexamide (add 5 ul of 10 mg/ml of CHX per ml of cytoplasm)
- DTT to a final concentration of 2 mM

11) MIX VERY WELL. Use a 25 ml pipette. THIS IS IMPORTANT!

12) Spin at either:
- 27K for 20 minutes in SW55
- 30K for 40 minutes in SW41 (this looks odd)

13) You should see a golden translucent cytoplasm with a discrete band of cloudy membranes floating in it (see picture below). Aspirate away remainder of yellow lipid from the top of the tube. Using a Pasteur pipette gently remove cytoplasm and membranes to a clean tube. Take care NOT to disturb the pellet.

14) Mix well by pipetting and snap freeze in lN2 in aliquots (usually 100 ul).