Preparation of CSF arrested extracts from Xenopus tropicalis 5/27/08
Protocol from Petr Kalab and Harland Lab

Priming & Boosting

- 16-24 hours before boosting, prime frogs by injection of 10 U of Chorulon HCG using a 30 gauge needle. Typically, inject 100 ul of 100 U/ml stock ("1:10 dilution") made in dilution buffer provided with the Chorulon hormone. Location of injection is on the back, outside of the stitch marks and near the butt.
- Store frogs under bench ON.
- 5 hours before extract, boost frogs by injection of 200 U of Chorulon HCG (200 ul of 1 U/ul, "1x" stock). Return to under bench but with light.
- "Squeeze" frogs 5 hours after boosting. By squeeze, just hold the frog in your hand and let them struggle. Both laid eggs and squeezed eggs are good for extract. Expect 100-200 ul of extract/frog, so use a minimum of 4 frogs/extract.

Extract
All steps of the extract are exactly the same as with laevis (Petr's protocol) except for:

1. The dejellying process.
2. The extract should NEVER get colder than 16C. NEVER ON ICE.
3. You must add cytoD to the eggs before the crushing spin.
4. The 1st mitosis is better than CSF. So it's ok if they exit to interphase.

Dejellying Solution	**CSF-XB, pH 7.7-7.8**	**CSF-XB+**	**Beckman SW50 tube**
(3% cysteine, pH 7.8-8.0)	50 ml XB salts	50 ml CSF-XB	1 ml CSF-XB+
15 g L-cysteine	25 ml 2M sucrose	50 ul LPC	**10 ul cytoD**
500 ml dH20	10 ml 1M HEPES pH 7.7		
pH with NaOH	1 ml 2M MgCl2		
Make fresh – right before use!	10 ml 1M K-EGTA		
	dH20 to 1L		
	pH with KOH		
	Keep at RT		
	Store at 4C for < 6 mos		

Protocol

Collect eggs with a transfer pipette and put in plastic beaker.
Pour off as much water as possible and add dejellying solution.
Swirl and exchange dejellying solution until see coats come off and eggs rotate with black pole up. If eggs don't dejelly in about 10 minutes, the eggs may be bad.
Pour off as much dejellying solution as possible without exposing eggs to air.
Pour in CSF-XB and swirl. Repeat using about 400-500 ml total.
Repeat with CSF-XB+.
Load eggs with cutoff plastic pipette into SW50 tubes, never exposing eggs to air.
Place SW50 tubes into Sarstedt tubes using forceps.
Packing spin the tabletop centrifuge:
1' 1600 rpm
Remove as much buffer as possible, sacrificing eggs if necessary, with a plastic pipette.
Spin in HB-6 rotor at 10200 rpm between 16C and 20C for 16' (17' on dial, rotor code 07).
Remove cytoplasmic layer with 1 ml syringe and 18g needle.
Expel extract into eppendorf in 16C bath.

Add LPC (1:1000), cytoD (1:500), and energy (1:50).

Cycle
To enter interphase, add CaCl2 to 500 mM (~ 45').
To enter mitosis, add same volume of CSF extract (~30').

Clean up

- Keep frogs under bench for 24 hours to allow them to finish laying and check for lesions. Then return to return tank in room 30A.
- Clean 6L plastic tubs with 10% bleach solution. Do NOT use soap or detergent.

CHEAT SHEET

Prime with 100 ul of 1:10 dilution HCG.
Boost with 200 ul of 1x HCG.
Squeeze 5 hrs later / make CSF-XB and +.
Collect eggs.
Make cysteine soln.
Dejelly eggs.
CSF rinses.
Pack 1' 1600 rpm.
Spin 15' 10200 rpm.
Draw and add cytoD 1:500 and LPC 1:1000 and energy.
Store at RT