

Laevis Extract Prep from Blake Riggs, Heald Lab 1/29/08

Dejelling Solution (2% cysteine in 1x XB pH 7.8) 5 g L-cysteine 12.5 ml 20x XB salts 1 ml 10N NaOH 237 ml dH2O Make fresh – right before use!	XB 25 ml XB salts 12.5 ml 2M sucrose 5 ml 1M HEPES pH 7.7 65 ul 10N KOH 458 ml dH2O Make on day of use - keep at 16C	CSF-XB 200 ml XB 100 ul 2M MgCl2 2 ml 0.5M K-EGTA Make on day of use – keep at 16C
CSF-XB+ 50 ml CSF-XB 50 ul LPC Make on day of use – keep at 16C	Beckman SW50 tubes 2 per frog 1 ml CSF-XB+ 10 ul cytoD Make fresh – right before use	Spindle Fix 600 ul 80% glycerol 300 ul 37% formaldehyde 50 ul 20x MMR 1 ul Hoechst Keep in dark RT. Good for a few days.
15 ml Sarstedt tubes 1 ml dH2O	50x Rhodamine-tubulin 2 ul aliquot from –80C 8 ul extract Mix gently. Keep on ice.	20x Sperm nuclei 3 ul sperm aliquot from –80C 27 ul cold XB Mix well by pipetting roughly. Keep on ice.

Protocol

Inject females with 500 U HCG (500 ul of 1U/ul) 16 hours before use.
 Collect eggs in 1x MMR and wash with 1x MMR if crap in water.
 Pour off as much MMR as possible and add dejelling solution.
 Swirl and exchange dejelling solution until see coats come off and eggs settle.
 Pour off as much dejelling solution as possible without exposing eggs to air.
 Pour in XB and swirl. Save 1 ml of XB in an eppendorf tube.
 Repeat with XB, CSF-XB, CSF-XB+.
 Load 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' 800 rpm
 30" 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 15' (16' on dial, rotor code 07).
 Remove SW50 tubes and place them directly on ice.
 Remove cytoplasmic layer with 1 ml syringe and 18g needle.
 Expel extract into eppendorf on ice.
 Add LPC (1:1000), cytoD (1:500), and energy (1:50).
 Clarifying spin at 13200 rpm for 10' at 4C (in cold room).

Checking reaction:

20 ul extract
 1 ul 20x sperm nuclei
 0.5 ul 50x rhodamine tubulin

Squash 1 ul with 5 ul spindle fix.

In 30-45' should have half spindles. In 60' should have bipolar spindles.