Laevis Embryo Extract Protocol (adapted from Jeremy Wilbur)
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Dejellying Solution **(2% cysteine in 1x XB pH 7.8)** 1 g L-cysteine 200 µl 10N NaOH 30 ml dH20 Make fresh – right before use!	**CSF-XB+** 2 mls 10X XB 200 µl 0.5M K-EDTA 10 µl 2M MgCl2 10 µl LPC 17.8 ml dH20 Make fresh – right before use!	**XB** Make 10-30 ml 1X XB from 10X stock

Protocol

In vitro fertilization

1. Inject females with 500 U HCG (500 ul of 1U/ul) 16 hours before use.
2. Squeeze the female(s) atop the petri dish(es) to catch the eggs
    - Use petri dish coated with 1.5% agarose in 1/10MMR to avoid eggs sticking to glass and help in forming a monolayer
    - Try and get all of the eggs you need (100-200 eggs per dish) on the first
    - If any liquid, remove using a transfer pipet or by blotting with paper towels
3. Transfer the testes in 1 mL of ddH2O, cut with scissors in tiny pieces and crush with a 1.5 mL pestle
4. Transfer all 1 mL of testicle solution atop the eggs per petri dish, try to expel the liquid within the egg mass do facilitate the formation of monolayer
5. Gently agitate the dish to form as much as possible a monolayer of eggs
6. Incubate for 10 min RT (briefly and gently agitate the dish after 5 min)
7. Gently flood the dish with a few milliliters of 1/10 MMR and briefly swirl the dis
8. Transfer the petri dish to the 23°C incubator

After Fertilization

9. Approximately 25 min after fertilization, dejelly with 1g of cysteine, 200 µL of NaOH 10N in 30 mL of ddH2O
10. Replace with fresh dejelly solution 1-2 times
11. Gently buffer exchange 3-4 times with 1/10 MMR
12. Transfer the dishes back to the 23°C incubator
13. After the first cell division (approx. 1.5 hours post-fertilization) edit out ALL unfertilized eggs and wait until desired stage to make extract.

4 cell stage-start making extract at 1hr 45min post fertilization. (~41um spindles)
stg 7 – 4.5hrs after fertilization (~20um spindles)
stg 8 – 5.5hrs after fert has ~12-15um spindles

Making Extract

14. With a disposable transfer pipette, transfer embryos into a 2ml eppendorf tube
15. Wash embryos several times with XB
16. Wash embryos several times with CSF-XB+
17. For final wash with CSF-XB+, add cyto-D 1:500
18. Remove as much excess buffer as possible using a p200 pipette
19. Pack embryos with a packing spin in table top microfunge by spinning at 1000rpm for 1min then 2000 rpm for 30 secs
20. Remove as much buffer as possible using a bent p200 pipette tip or gel-loading pipette tip.
21. Crush embryos by spinning in HB-6 rotor at 10200 rpm between 16C and 20C for 12 min' (rotor code 07, use green tube adaptors). Place directly on ice after spin

22. Remove lipid layer by aspiration or poke hole in the lipid layer to access cytoplasmic fraction using a p200 pipette.
23. Remove the cytoplasmic layer using a p200 pipette. (4 cell/early stages clearish/golden, Stg 8 later stages often contains black pigment/Ribosomes – also contain lots of nuclei).
24. Expel extract into eppendorf on ice.

- Can also filter extract through a 1.0 µm syringe filter to remove endogenous nuclei but will lose a significant amount of extract volume.

25. Add LPC (1:1000), cytoD (1:500), and energy (1:50).
26. To make spindles add:
1:100 final conc. Cyclin B delta 90
1:100 ~14.6 mg/ml UbcH10 C117S dominant negative mutant
400X Rhodamine tubulin
200X Sperm
bring to room temperature
spindles will form in 45-90 minutes