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Preparation of Cellular Extracts from *Xenopus* Eggs and Embryos

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Abstract

Cell-free cytoplasmic extracts prepared from *Xenopus* eggs have been used extensively to recapitulate and characterize intracellular events in vitro. Egg extracts can be induced to transit the cell cycle and reconstitute assembly of dynamic structures including the interphase nucleus and the mitotic spindle. In this protocol, methods are described for preparing crude cytoplasmic extracts from *Xenopus* eggs and embryos that are arrested in metaphase of the cell cycle. The basic protocol utilizes unfertilized *Xenopus laevis* eggs, which are crushed by centrifugation in the presence of EGTA to preserve the natural Cytostatic Factor (CSF) activity that maintains high levels of Cdk1/cyclin B kinase and metaphase arrest. In the second method, the basic procedure is adapted for *Xenopus tropicalis* eggs with minor modifications to accommodate differences in frog size, timing of egg laying, and temperature and salt sensitivity. The third variation takes advantage of the synchronous divisions of fertilized *X. laevis* eggs to generate extracts from embryos, which are arrested in metaphase by the addition of non-degradable cyclin B and an inhibitor of the anaphase-promoting complex (APC) that together stabilize Cdk1/cyclin B kinase activity. Because they are obtained in much smaller amounts and their cell cycles are less perfectly synchronized, extracts prepared from embryos are less robust than egg extracts. *X. laevis* egg extracts have been utilized to study a wide range of cellular processes. In contrast, *X. tropicalis* egg extracts and *X. laevis* embryo extracts have been used primarily to characterize molecular mechanisms regulating spindle and nuclear size.

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RECIPES

CSF-XB+ (for 100 mL)

100 mL CSF-XB

100 μl LPC

Prepare fresh and use immediately.

CSF-XB+ solution #2 for X. tropicalis eggs (for 50 mL)

50 mL CSF-XB Solution #2

50 μl LPC

Prepare fresh and use immediately.

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MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Agarose (1.5% in 0.1x MMR)

Microwave before using to coat the bottom of a petri dish. Dishes should be prepared the day before embryo extract preps, covered with 0.1x MMR and stored at RT. Discard the 0.1x MMR before use.

CSF-XB <R>

This buffer is used for X. laevis eggs and embryos.

CSF-XB+ (CSF-XB with 1 μg/mL LPC) <R>

This buffer is used for X. laevis eggs and embryos. Make fresh on day of extract preparation.

CSF-XB solution #2 for *X. tropicalis* eggs <R>

This buffer is used for X. tropicalis eggs and contains additional EGTA and magnesium chloride.

CSF-XB+ solution #2 (CSF-XB solution #2 supplemented with 1 μg/mL LPC) <R>

This buffer is used for X. tropicalis eggs and contains additional EGTA and magnesium chloride. Make fresh on day of extract preparation.

Cysteine (Sigma C-7352, or other source)

Cytochalasin D or B (10 mg/mL, Sigma C8273 or Sigma C6762, respectively)

Dissolve in DMSO and store in 50 μl aliquots at −20ºC.

Dejelly solution #1 for *Xenopus laevis* eggs <R>

Dejelly solution #2 for *Xenopus tropicalis* eggs and *Xenopus laevis* embryos <R>

Unlike the X. laevis dejelly solution, the X. tropicalis buffer does not contain salts.

Dimethyl Sulfoxide (DMSO) (anhydrous, high purity such as Sigma)

EGTA (K salt; 0.5 M) (pH 7.7)

Filter sterilize and store in 2 mL aliquots at −20ºC.

Energy mix (50x) <R>

Note that some labs do not add energy mix to egg extracts.

Extract Buffer salts (20x XB salts) <R>

Autoclave and store at 4ºC.

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HEPES (K salt; 1M; pH 7.7)

Filter sterilize and store in 5 mL aliquots at −20°C.

Human chorionic gonadotropin (HCG) (Sigma CG10) or chorulon (A to Z Vet Supply), at a concentration of 1000 IU/mL

Dissolve in sterile MilliQ H_2O and store at 4°C.

LPC protease inhibitor cocktail (10 mg/mL each of leupeptin, pepstatin, chymostatin) (EMD Millipore)

Dissolve in DMSO and store in 50 µL aliquots at −20°C.

Marc's Modified Ringer's (MMR; 20x) <R>

Dilute to desired concentration with MilliQ H_2O before use.

Mature female Xenopus laevis or Xenopus tropicalis frogs

Frogs are obtained from NASCO. Females are used to obtain eggs as described below.

Mature male *Xenopus laevis* frogs

Frogs are obtained from NASCO. Males are sacrificed to obtain fresh testes for fertilization to obtain embryos (see Protocol: Isolation and demembranation of Xenopus sperm nuclei<prot099044> [Hazel and Gatlin 2018)].

$MgCl_2$ solution (2M)

Pregnant mare serum gonadotropin (PMSG; 200 IU/mL; Prospec HOR-272)

Dissolve in sterile MilliQ H_2O and store at 4°C.

Purified proteins: Aliquots of 0.5 mg/mL (~ 13 µM) Δ90-CyclinB1 in XB (Glotzer et al., 1991); 15 mg/mL (~ 750 µM) human UbcH10-C114S in XB (Rape et al., 2006)

Sucrose (2M)

Filter sterilize and store in 12.5 mL aliquots at −20°C.

XB: extract buffer <R>

Equipment and Containers

Centrifuge (floor size, high speed, refrigerated) set to16°C

Centrifuge tube (round-bottom 13 mL polypropylene 16.8 × 95 mm; Sarstedt 55.518)

Clinical centrifuge (refrigerated, swinging-bucket) set to either 16°C or 25°C.

Beakers, plastic (0.5 L and 4 L) and glass (500 mL)

Dissection stereomicroscope

Forceps

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Ice bucket with ice

Microcentrifuge tubes (1.5 and 2 mL)

Petri dishes (glass, 6 cm diameter)

Plastic containers for frogs (4 L and 6 L; Corning) with tight-fitting lids and holes punched for air exchange

Plastic pestle for 1.5 mL microfuge tube (USA Scientific)

Room (16°C) or large non-air tight incubator set to 16°C for *Xenopus laevis*

Rotor (swinging bucket Sorvall HB-6) with rubber (Kimble-Chase) and microcentrifuge tube adapters (Sorvall)

Syringes (disposable; 1 mL)

Syringe needles (18-gauge 1.5” length, and 30-gauge, 0.5” length)

Transfer pipettes (plastic, draw up to 3.4 mL per squeeze; Fisher)

Ultracentrifuge tubes (SW-55 ultra-clear thin-wall 5 mL; 13 × 51 mm, Beckman 344057)

METHODS

Three different extract preparation procedures are outlined, which have been optimized for *X. laevis* eggs, *X. tropicalis* eggs, or *X. laevis* embryos.

Xenopus laevis Egg Extract Procedure

1. Prime *Xenopus laevis* females at least 3 days prior to extract preparation by injecting 0.5 mL (100 IU) of PMSG subcutaneously into the dorsal lymph sac using a 30-gauge needle and 1 mL syringe.

   Typically, 3–4 females are used for each extract preparation. Priming the frogs increases egg yield and egg quality. Primed frogs should be used within two weeks. Depending on hormone source, the injected amount may need to be titrated. If the frogs lay eggs following priming, subsequently reduce the amount injected by 50%.

2. Induce ovulation by injecting primed frogs 16–18 hours prior to extract preparation time with 0.5 mL (500 IU) of HCG. Store each frog individually in 2 L of 1x MMR in a 4 L plastic container overnight at 16°C. On average, each female should lay more than a thousand eggs (Figure 1A).

   Housing frogs individually prevents mixing of egg clutches of variable quality.

3. Remove frogs from containers and analyze egg quality. Good eggs have clearly delineated animal (dark) and vegetal (light) poles. Remove lysed (white and

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puffy), mottled or abnormal looking eggs from containers using a transfer pipette. Store eggs at 16°C until all buffers and supplies are prepared.

Clutches that contain a significant number of eggs (>2%) that are activated or lysed (white and puffy appearance) or connected in long strings should not be used.

4. For eggs from 3–4 females, prepare 1 L Dejelly solution #1, 1 L XB, and 400 mL CSF-XB. Ten minutes prior to the extract prep, prepare 100 mL CSF-XB+, dispense 1 mL CSF-XB+ to 4 ultracentrifuge tubes, and add 10 μl of cytochalasin D (final concentration of 100 μg/mL) to each tube. For supplies: trim tips from 4 transfer pipettes to increase opening diameter to ~2 mm, and leave 4 uncut. Gather 4 L plastic and 500 mL glass beakers and all required reagents and supplies. Make sure that clinical and high-speed centrifuges are set to 16°C.

One frog typically produces enough eggs to fill one tube.

It is crucial to be prepared for all subsequent steps before starting.

Dejelly and egg-washing steps can be performed at room temperature but extract quality may be higher if steps are carried out in a 16°C room.

5. In the next steps (6–8), gently pour buffers down the side of the beaker, and swirl eggs while avoiding excess turbulence. Between steps, pour off as much buffer as possible into a 4 L beaker without exposing the eggs to air, which will lyse them. Manipulate eggs carefully and once eggs are dejellied, carry out subsequent washing and centrifugation steps in rapid succession. Throughout the procedure, remove abnormal or lysed eggs with a transfer pipette.

6. Clean the eggs: Pour off 1x MMR from plastic container containing laid eggs. Gently pour in 250–500 mL fresh 1x MMR and repeat washes until all dirt and debris are removed.

7. Dejelly eggs: Combine best clutches of eggs into a single 500 mL glass beaker and pour off remaining MMR. Add ~250 mL of Dejelly solution #1. Swirl the eggs gently, every 20 seconds, and every 2 minutes pour off and replenish the solution. Dejellying is complete once eggs pack together tightly, without gaps (Figure 1B). The close packing is easiest to discern visually when beaker is tipped at a 45° angle. Total time to dejelly will vary, but should be around 5 minutes. Once completed, pour off the dejelly solution and immediately add ~300 mL of XB.

Alternatively, each egg clutch can be transferred to an individual beaker and dejellied and washed separately using smaller buffer volumes.

8. Wash eggs: Pour off the first addition of XB and wash again with 300 mL. Pour off the XB and wash two times with ~200 mL of CSF-XB. Pour off the CSF-XB and add 100 mL of CSF-XB+. At this point the eggs should appear very clean with their dark animal poles facing up. Sort one final time to remove any lysed or abnormal eggs.

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9. Transfer eggs very carefully to prepared ultracentrifuge tubes containing CSF-XB+ and cytochalasin. Use a trimmed transfer pipette, and avoid exposing the eggs to air by first drawing up 0.5 mL of CSF-XB+ buffer and then gently drawing up the eggs, and expelling them below the liquid surface. Typically 2–3 fillings of the transfer pipette are required fill one tube with eggs (Figure 1C).

   Do not overfill tubes, which exposes eggs to air.

10. Packing spin: Using forceps, place each tube filled with eggs inside a 13 mL Sarstedt tube. Transfer tubes to clinical centrifuge at 16°C. Balance rotor and spin at 250 *g* for 1 min, and then at 500 *g* for an additional 30 sec to pack the eggs (Figure 1D). After spinning, remove all buffer from the top of the eggs using a transfer pipette.

    This step prevents buffer from diluting the extract, so it is worth sacrificing a few eggs to ensure that all buffer is removed.

11. Crushing spin: Transfer tubes filled with packed eggs to a Sorvall HB-6 rotor with rubber adapters in a high-speed centrifuge. Crush and fractionate the eggs by spinning at 18,000 *g* for 15 min at 16°C (Figure 1E).

12. Collect cytoplasm: Immediately upon completion of centrifugation, remove thin-wall tubes contained fractioned eggs from Sarstedt tubes and place them on ice. Using an 18-gauge needle attached to a 1 mL syringe, puncture the thin-wall tube at the bottom of the cytoplasmic layer (Figure 1F). Aspirate the cytoplasm without taking up any of the surrounding yolk or lipid layers. Remove the needle and expel the cytoplasm into a pre-chilled tube on ice.

    To avoid piercing your finger and to keep the tube cool while withdrawing the extract, hold the tube near the top up against the inside of the ice bucket, keeping the bottom of the tube on ice.

    It is good to withdraw some of the fluffy whitish layer underneath the cytoplasmic layer as well, which can be achieved by rotating the needle so that the opening is facing downward.

    Expected yield is ~1 mL of crude cytoplasm from each tube.

13. Supplement the extract with a final concentration of: 10 μg/mL LPC (1:1000 dilution of stock), 10 μg/mL cytochalasin D (1:1000 dilution of stock). Add Energy mix stock at 1:50. Mix by inverting the microfuge tube or pipetting gently with a cut off tip. The freshly prepared extract can be used for 6–8 hours.

    The final molar concentrations of reagents supplemented to the extract from this step are: 16.67 μM LPC, 20 μM Cytochalasin D, 3.8 mM creatine phosphate, 0.5 mM ATP, 0.5 mM MgCl2 and 0.05 mM EGTA. Not all labs supplement the extract with energy mix.

Xenopus tropicalis Egg Extract Procedure

Major differences from X. laevis egg extract preparation accommodate the smaller frog size, its higher physiological temperature, and sensitivity of the eggs to salt.

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1. Dilute HCG stock to 100 IU/mL in sterile MilliQ water and prime *X. tropicalis* females 20–21 hours prior to boosting by injecting 250 μL (25 IU) HCG subcutaneously into the dorsal lymph sac using a 30-gauge needle and 1 mL syringe. Store frogs in 6 L plastic container filled with 4 L of salt water.

   Typically, 6–8 females are used for one extract preparation with a yield of 0.1 – 0.2 mL cytoplasm per egg clutch.

   Larger tanks are used for X. tropicalis to facilitate squeezing and because they tend to jump.

   If working with both X. laevis and X. tropicalis, always use dedicated containers for each species.

2. Induce ovulation by boosting primed frogs ~4 hours prior to extract preparation by injecting 250 μL (250 IU) HCG. To control for egg quality make sure to separate frogs into individual containers filled with deionized water.

   Note that the time from boosting injection to ovulation is much shorter than for X. laevis.

3. Once the first eggs are laid, 2.5–3 hr following the second injection, accelerate egg laying by gently clasping frogs to mimic amplexus every 15 min. Females will wiggle causing further egg laying. After 4–5 ‘squeezes’, analyze egg quality. Good eggs have clearly delineated animal (dark) and vegetal (light) poles.

   Stringy eggs are okay for X. tropicalis. Clutches of eggs that are activated or lysed (white and puffy appearance) should not be used.

4. Prepare buffers and set up equipment during the clasping period. For eggs from 6–8 females, prepare 0.5 L Dejelly Solution #2, and 1 L CSF-XB solution #2. Ten minutes prior to the start of experiments, prepare 50 mL CSF-XB+ solution #2, and dispense 1 mL CSF-XB+ solution #2 supplemented with 10 μl of 10 mg/mL cytochalasin D into a single ultracentrifuge tube. For supplies: trim tips from 4 transfer pipettes to increase opening diameter to ~2 mm, and leave 4 uncut, gather 4 L and 500 mL plastic beakers, and all required reagents and supplies. Make sure that clinical centrifuge is set to 25°C and high-speed centrifuge is set to 16°C.

5. In the next steps (19–21), gently pour buffers down the side of the beaker, and swirl eggs gently to avoid turbulence. Between steps, pour off as much buffer as possible into a 4 L beaker without exposing the eggs to air, which will lyse them. Manipulate eggs carefully and once eggs are dejellied, carry out subsequent washing and centrifugation steps in rapid succession. Throughout the procedure, remove abnormal or lysed eggs with a transfer pipette.

6. Remove the frogs from the containers. Detach the eggs stuck to the container by gently sweeping them with your gloved finger or by generating water flow using a transfer pipet to put them back into suspension in the water. Before they settle and stick again, transfer them to a single 500 mL plastic beaker. Combine the best clutches into a single 500 mL plastic beaker.

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Note that eggs stick to some glove brands.

7. Dejelly eggs: pour off remaining water. Add ~250 mL of Dejelly solution #2. Swirl the eggs gently, every 20 sec, and after 2 min pour off and replenish dejelly solution. Dejellying is complete once eggs pack together tightly, without gaps, when beaker is tipped to a 45° angle. Total time to dejelly will vary but should take less than 10 min. Once completed, pour off the dejelly solution and immediately add 250 mL CSF-XB solution #2.

8. Wash eggs: Pour off the first addition of CSF-XB solution #2 and wash several more times with ~250 mL CSF-XB solution #2. Pour off as much buffer as possible and add 50 mL of CSF-XB+ solution #2. At this point the eggs should be clean and their dark animal poles should be facing up. Sort one final time to remove any bad eggs.

9. Transfer eggs very carefully to prepared ultracentrifuge tube containing CSF-XB + solution #2 and cytochalasin. Use trimmed transfer pipette, and avoid exposing the eggs to air by first drawing up 0.5 mL of CSF-XB+ buffer and then gently drawing up the eggs, and expelling them below the liquid surface.

   A single tube can accommodate eggs from ~ 6 females, and to avoid dilution it is better not to divide eggs into two tubes.

10. Packing spin: Using forceps, place ultracentrifuge tube filled with packed eggs inside a Sarstedt tube. Transfer to a refrigerated clinical centrifuge set to 25°C. Balance rotor and spin at 500 *g* for 1 min, to pack the eggs. After spinning, remove all buffer from the top of the eggs using a transfer pipette.

    This step prevents buffer from diluting the cytoplasm, so it is worth sacrificing a few eggs to ensure all buffer is removed.

11. Crushing spin: Transfer tube filled with packed eggs and balance tube to high-speed centrifuge containing a Sorvall HB-6 rotor with rubber adapters. Crush and fractionate the eggs by centrifuging at 18,000 *g* for 15 min at 16°C.

12. Collect cytoplasm: Upon completion of centrifugation remove ultracentrifuge tubes from Sarstedt tubes and place them at room temperature or 16°C. Using an 18-gauge needle attached to a 1 mL syringe, puncture the thin-wall tube at the bottom of the cytoplasmic layer and carefully aspirate the cytoplasmic layer without taking up any of the surrounding yolk or lipid layers. Expected yield is ~ 1 mL of crude cytoplasm from one tube filled with eggs from 6 females. Remove the needle and expel the cytoplasm to 1.5 mL microfuge tube.

13. Supplement the extract with a final concentration of 10 μg/mL LPC (1:1000 dilution of stock), 10 μg/mL cytochalasin D (1:1000 dilution of stock). Add Energy mix stock at 1:50. Mix by inverting the microfuge tube or pipetting gently with a cut off tip. The freshly prepared extract should be kept at 16 °C and can be used for 5–6 hr.

    Note that unlike X. laevis egg extracts, X.tropicalis egg extracts should not be placed on ice, as this will compromise activity.

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Xenopus laevis Embryo Extract Procedure

Extracts arrested in mitosis can be prepared from X. laevis *embryos through blastula Stage 8, after which cell cycle synchrony is lost. The amount of extract that can be obtained from embryos is much lower than from eggs. Therefore, this procedure has not yet been applied* X. tropicalis*, due to its smaller egg size and lower yield.*

1. Prime *X. laevis* females 3–7 days prior to performing in vitro fertilization (IVF) by injecting 0.5 mL (100 IU) of PMSG subcutaneously into the dorsal lymph sac using a 30-gauge needle and 1 mL syringe.

2. Induce ovulation 14–15 hr prior to collecting embryos by injecting primed frogs with 0.5 mL (500 IU) HCG. Store each frog individually in 2 L of 1x MMR in a 4 L plastic container at 16°C overnight.

   Usually 3 out of 4 females will lay high-quality eggs, allowing the preparation of 3 separate single-frog embryo extracts.

3. Analyze egg quality 14 hr after HCG booster injection. Avoid using females that have laid lysed or stringy eggs, or that have laid no eggs at all.

4. Collect freshly-laid eggs: For selected frogs, promote egg-laying by gently squeezing females in a manner that mimics amplexus (Figure 2A), and collect freshly laid eggs from each frog in individual 6 cm glass dishes that have been coated with ~5 mL of 1.5% agarose in 0.1x MMR. Repeat every 15 min (up to 4 total squeezes) or until each dish is full. Place females in deionized water in between rounds of egg collection.

5. Prepare sperm slurry: For each IVF reaction, use 1/8 of a testis recently isolated from a *Xenopus* male (see Protocol: Isolation and demembranation of Xenopus sperm nuclei<prot099044> [Hazel and Gatlin 2018)). Use scissors to cut the testis into appropriately sized pieces, and place each in its own 1.5 mL microfuge tube containing 1 mL of MilliQ H_2O. Crush each piece using a plastic pestle for 1–2 min.

   Testes can be used for up to 1–2 weeks following dissection, but fertilization efficiency may drop over time.

6. Fertilize eggs: For each IVF reaction in a separate dish, pipet 1 mL of a slurry that contains approximately one eighth of a testis onto the eggs and incubate for 5–10 min at 16°C. Flood each dish with 0.1x MMR and incubate an additional 10–20 min or until all of the zygotes have rotated so that the animal cap (dark) is facing up (Figure 2B), indicating successful fertilization.

7. Dejelly eggs: This should be performed just after rotation, approximately 15 min post-fertilization, and prior to the first cleavage, which occurs at approximately 90 minutes. Pour off 0.1x MMR and add 20 mL of freshly prepared Dejelly solution #2 to each dish. Swirl gently for 2–3 min, until embryos pack tightly together when dish is tilted. Pour off Dejelly solution and wash 3–5 times with 0.1x MMR. Add 15 mL 0.1x MMR and continue incubation. Let embryo development progress at 16°C until the desired developmental state.

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At room temperature the first cytokinesis should take place ~ 90 min post fertilization.

At 16°C, embryos reach mid-Stage 4 at ~ 4.5 hr, or mid-Stage 8 at ~ 13.5 hr.

8. Sort embryos: Using a dissecting stereomicroscope, transfer embryos that are cleaving nicely to a fresh dish coated in 1.5% agarose in 0.1x MMR. Continue to monitor developmental progression by recording the timing of the second and third divisions. Remove embryos that are lagging or beginning to lyse.

9. Prepare remaining buffers and carry out final sort 30 min prior to appropriate timepoint for embryo collection. Prepare 0.2 L of XB solution, and 100 mL of CSF-XB+ supplemented with 100 μL of 10 mg/mL cytochalasin D. Perform final sort to ensure that the remaining ~ 1000 embryos in each dish are homogeneous.

10. Wash embryos: Pour off 0.1x MMR and add in succession to each dish: 2 × 25 mL of XB, then 2 × 15 mL of CSF-XB+ with cytochalasin. Gently swirl and pour off buffers between each addition. Tilt dish so that surface tension helps to carefully pull the solution over the lip of the dish. This ensures that embryos will not be poured off during buffer exchanges.

11. Pack embryos: After pouring off remaining buffer, and without exposing embryos to air, carefully transfer the embryos from a single dish into a 2 mL microfuge tube using a cut transfer pipette. A full dish of embryos, unpacked, will fill the tube to a level of ~ 1.7 mL (Figure 2C). Centrifuge at 200 *g* for 1 min and then at 500 *g* for 30 sec in a benchtop microcentrifuge at room temperature. Remove remaining buffer with a pipet, first using a P1000 and then a P200 tip.

12. Crush embryos: Transfer 2 mL tubes containing packed eggs to a Sorvall HB-6 rotor containing microfuge tube adapters, in a superspeed centrifuge set to 16°C. Centrifuge at 18,000 *g* for 12 min.

Alternatively, eggs can be crushed by spinning for 12 min at 18,000 g using a benchtop refrigerated microcentrifuge set to 16°C.

13. Collect embryo cytoplasm: After crushing the embryos into stratified layers, place the 2 mL microfuge tube on ice. Using a bent P200 pipette tip (Figure 2D), insert the tip from above, through the lipid layer and carefully withdraw the cytoplasm, making sure to avoid surrounding layers. Dispense the cytoplasm into a pre-chilled 1.5 mL microfuge tube. Expect a yield of ~ 400–500 μL of crude cytoplasm for each full plate of embryos. The layers correspond to, top: lipid (white-yellow), middle: cytoplasm (gold), bottom: yolk granules and pigment (black/brown) (Figure 2D).

14. Supplement the embryo extract with a final concentration of 10 μg/mL LPC (1:1000 dilution of stock) and 10 μg/mL cytochalasin D (1:1000 dilution of stock). Add Energy mix at 1:50. Mix by inverting the microfuge tube. To ensure that the extracts are synchronized and arrested in mitosis, add a non-degradable form of cyclin B along with a dominant-negative form of the ubiquitinating E2, UbcH10 (UbcH10-C114s) to final concentrations of ~0.35 μM Δ90-CyclinB1

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and ~18 μM UbcH10-C114S. Mix by inverting tube or pipetting gently with a cut off tip. Embryo extracts stored on ice are functional for up to 6 hr.

To further enhance metaphase arrest and improve spindle forming activity, the embryo can be supplemented with 5–10% CSF-arrested cytoplasmic egg extract from Xenopus laevis (Good, 2016).

DISCUSSION

These procedures are based on the original method first described by Lohka and Masui (Lohka and Maller, 1985; Lohka and Masui, 1983), optimized initially by Andrew Murray (Murray, 1991) and further refined over the years (Desai et al., 1998; Good, 2016; Maresca and Heald, 2006). Central to the success of all extract preparations is the quality of the eggs. Crude or further fractionated *Xenopus laevis* egg extract have been used widely for a variety of protocols. A major strength of in vitro systems such as these comes from the ability to manipulate them biochemically by immunodepletion or by adding recombinant or fluorescent probes.

Xenopus tropicalis frogs are considerably smaller than their *X. laevis* relative, and lay proportionally smaller eggs. Therefore, they are less suitable for generating the large volumes of egg extract that form the basis of many assays. However, the observation that the size of nuclei and spindles formed in *X. tropicalis* egg extract are smaller than those formed in *X. laevis* and recapitulate in vivo differences has uniquely enabled investigation of mechanisms of intracellular scaling between species (Brown et al., 2007).

Extracts prepared from *X. laevis* embryos must be arrested in metaphase using exogenously added regulators of the cell cycle machinery. Cyclin B delta 90 is a non-degradable version of cyclin that that activates Cdk1 activity (Glotzer et al., 1991). Mitotic arrest is further enhanced by addition of UbcH10-C114S, which functions as a dominant negative inhibitor of the Anaphase Promoting Complex (APC) (Rape et al., 2006; Townsley et al., 1997). These extracts have been used to compare spindle morphologies between meiosis and the early mitotic divisions (Wilbur and Heald, 2013; Wuhr et al., 2008), and formed the basis of assays to examine spindle scaling during development (Good et al., 2013; Wilbur and Heald, 2013; Wuhr et al., 2008). Embryo extracts have also been prepared that are arrested in interphase that enable investigation of nuclear scaling (Levy and Heald, 2010). Like egg extracts, embryo extracts largely recapitulate in vivo size differences, but can be prepared only in very small amounts. Their activity is therefore reduced compared to egg extracts, likely due in part to increased dilution of the cytoplasm with buffer.

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REFERENCES

Brown KS, Blower MD, Maresca TJ, Grammer TC, Harland RM, and Heald R (2007). Xenopus tropicalis egg extracts provide insight into scaling of the mitotic spindle. The Journal of cell biology 176, 765–770. [PubMed: 17339377]

Desai A, Murray A, Mitchison TJ, and Walczak CE (1998). The Use of Xenopus Egg Extracts to Study Mitotic Spindle Assembly and Function in Vitro. Methods Cell Biol 61, 385–412.

Glotzer M, Murray AW, and Kirschner MW (1991). Cyclin is degraded by the ubiquitin pathway. Nature 349, 132–138. [PubMed: 1846030]

Good MC (2016). Encapsulation of Xenopus Egg and Embryo Extract Spindle Assembly Reactions in Synthetic Cell-Like Compartments with Tunable Size. Methods Mol Biol 1413, 87–108. [PubMed: 27193845]

Good MC, Vahey MD, Skandarajah A, Fletcher DA, and Heald R (2013). Cytoplasmic volume modulates spindle size during embryogenesis. Science 342, 856–860. [PubMed: 24233724]

Hazel JW, Gatlin JC. 2018 Isolation and demembranation of Xenopus sperm nuclei. Cold Spring Harb Protoc doi:10.1101/pdb.prot099044.

Levy DL, and Heald R (2010). Nuclear size is regulated by importin alpha and Ntf2 in Xenopus. Cell 143, 288–298. [PubMed: 20946986]

Lohka MJ, and Maller JL (1985). Induction of nuclear envelope breakdown, chromosome condensation, and spindle formation in cell-free extracts. The Journal of cell biology 101, 518–523. [PubMed: 3926780]

Lohka MJ, and Masui Y (1983). Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. Science 220, 719–721. [PubMed: 6601299]

Maresca TJ, and Heald R (2006). Methods for studying spindle assembly and chromosome condensation in Xenopus egg extracts. Methods Mol Biol 322, 459–474. [PubMed: 16739744]

Murray AW (1991). Cell Cycle Extracts. Methods Cell Biol 36, 581–605. [PubMed: 1839804]

Rape M, Reddy SK, and Kirschner MW (2006). The processivity of multiubiquitination by the APC determines the order of substrate degradation. Cell 124, 89–103. [PubMed: 16413484]

Townsley FM, Aristarkhov A, Beck S, Hershko A, and Ruderman JV (1997). Dominant-negative cyclin-selective ubiquitin carrier protein E2-C/UbcH10 blocks cells in metaphase. Proceedings of the National Academy of Sciences of the United States of America 94, 2362–2367. [PubMed: 9122200]

Wilbur JD, and Heald R (2013). Mitotic spindle scaling during Xenopus development by kif2a and importin alpha. Elife 2, e00290. [PubMed: 23425906]

Wuhr M, Chen Y, Dumont S, Groen AC, Needleman DJ, Salic A, and Mitchison TJ (2008). Evidence for an upper limit to mitotic spindle length. Current biology : CB 18, 1256–1261. [PubMed: 18718761]

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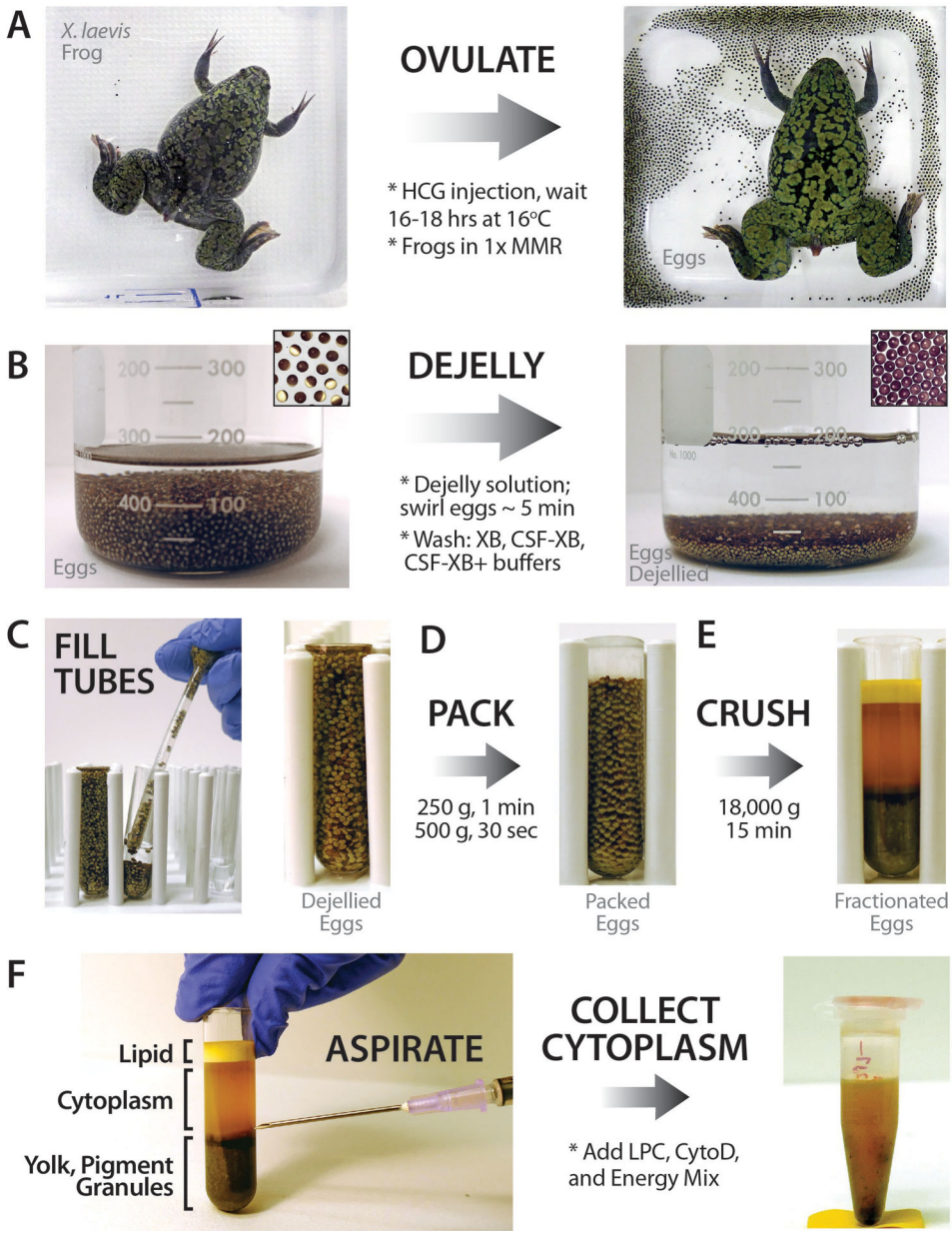

Figure 1 –.

Xenopus laevis egg extract preparation

A. Female frogs induced to ovulate by injection with HCG. Laid eggs are collected 16–18 hr after the injection. B. Eggs are dejellied using a cysteine solution. The eggs pack tightly together once their jelly coats have been removed. Inset: egg spacing before and after dejelly. C. Dejellied eggs are carefully transferred to an ultracentrifuge tube using a plastic transfer pipette whose tip has been cut to create a wider bore. D. A series of low-speed spins tightly packs eggs without lysing them, allowing excess buffer to be removed. E. A high-speed spin crushes and fractionates the eggs. F. The cytoplasm layer is collected by puncturing the tube wall using a needle and syringe. Once aspirated, the cytoplasm is transferred to a new tube and supplemented with additional reagents.

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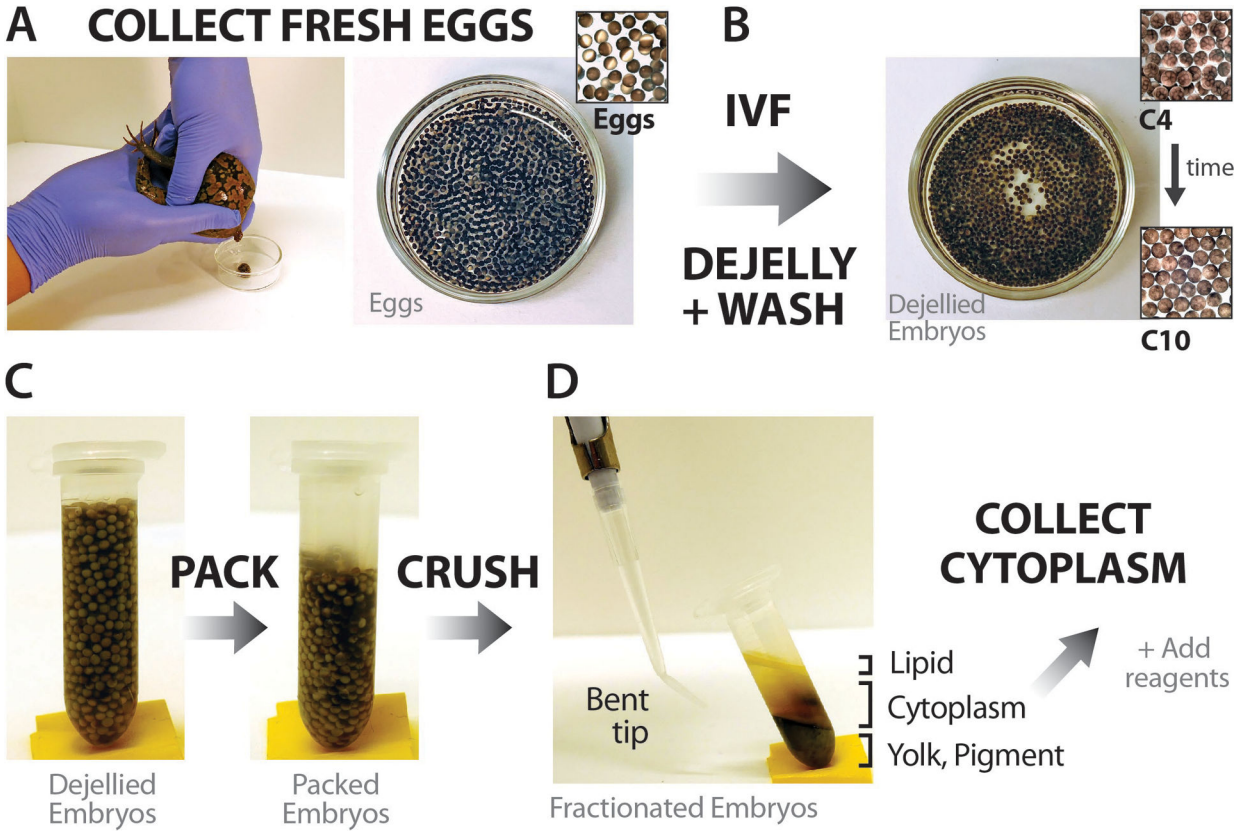

Figure 2 –.

Xenopus laevis embryo extract preparation

A. In contrast to egg extracts which can be prepared from laid eggs, embryo extracts require freshly squeezed eggs. B. Eggs are fertilized by sperm *in vitro* and subsequently dejellied prior to the first embryonic division. Embryos are synchronized and collected at specific developmental stages. Inset shows embryos that have undergone 4 cell divisions (C4) or 10 divisions (C10). C. Dejellied embryos are packed tightly together by centrifugation to remove excess buffer. These packed embryos are then crushed and fractionated by high-speed centrifugation. D. The cytoplasm layer is collected by threading a bent pipette tip through the lipid layer, and supplemented with reagents to generate the final crude embryo cytoplasm.