C. elegans embryo purification and embryo extract generation:

Aim: Collect *C. elegans* embryos from worms grown on plates. These embryos can be any stage that already has an eggshell, thus all the eggs that are **a)** present on the plate (old eggs) **b)** are still inside their mothers (early staged). Scale the protocol according to the amount of embryo mass required. This protocol should give around 500 mg of embryos.

Note: This protocol can yield up to 1 g of pure embryos. However there are many steps where caution and timing is important. For instance controlling the growth of the worm culture to time purification to the right moment is essential. Another problematic step is the bleaching. Sometimes it is better to not bleach a second time but instead filter the embryo solution using a sieve.
Last but not least, this protocol can be scaled to everyone's needs, but it is highly recommended to run a initial test first to learn the handling before attempting an upscaling.

Reagents and Tools:

- 10x 10 cm petri dishes with NGM + OP50 bacteria (standard worm growing plates)
- 40x 10cm Petri Dishes with 3.5% Agar + 2% Peptone + C600 (bacteria)
- Ice Cold M9 (3L), for 1L use: 3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 ml 1 M $MgSO_4$, rest H_2O
- Bleaching Solution: 8 parts H_2O, 1 part NaOCl (Sigma), 1 part 4M NaOH,
- < 40 µm sieves like the BD 352340 Falcon Blue nylon Mesh Cell strainer

Preparing C600 plates:

1. Pre-dry the plates (3.5% Agar + 2% Peptone) under the fume hood for 30 minutes with open lids.
2. Seed 3 ml C600 bacteria culture onto each plate.
3. Grow the bacteria culture on plates for 1.5 - 2 days at 30°C in an incubator.
   Note: Keep the petri dishes closed to prevent further evaporation!
4. Once the bacteria lawn is thick and dense plates are ready and can be stored at 4°C for later use.

Growing and synchronizing Worms:

1. Create 10x 10cm plates with starved *dauer* worms on. To do so transfer worms from a fully grown single 10 cm OP50 plate onto 10x 10cm OP50 plates and let them grow for 3 days. Transfer can be done by washing or by chunking.
2. Collect the starved *dauer* worms by washing them off the plates with 1x M9.
   Note: If worms have dug into the agar, let the M9 stay on the plate for 15-20 minutes because worms will then come into solution.
3. Distribute M9 + Worms into 50 ml Falcon Tube
4. Spin down worm soup at ~600 xg for 3 min.
5. Aspirate M9 and add fresh M9.
6. Distribute worm soup equally onto 30 x 10cm C600 plates and grow them for 2-3 days until all worms have developed into adults and started laying eggs.
7. Once the plates are full with adult worms they are ready for bleaching.
   Note: If you are unsure, better to harvest a bit too early then too late.

Bleaching Worms to extract embryos:

1. Wash worms off the plates. Add enough M9 to each plate to cover it completely in liquid and let stand for a while to allow worms to crawl into solution.
   Note: To reduce total amount of worm soup obtained you can transfer the M9 from one plate to the next.
2. Transfer worm soup into 50 ml Falcon tubes.
3. Spin down worm soup at ~600 xg for 3 min.
4. Aspirate M9 and add fresh M9.
5. Repeat step 4 to 5 two times to wash the worms.
6. In the last wash step pool all worms before adding fresh M9. Spin again at ~600 xg for 3 min

and aspirate M9. → You obtain a lose worm pellet

7. Distribute the worm pellet equally into 50 ml falcons such that each falcon contains not more than 5 ml worm soup.
8. For each 50 ml Falcon prepare 2x 50 ml Falcon tubes containing 35 ml M9. These will be used to quickly stop the bleaching reaction. Keep on ice.
9. Prepare fresh bleaching solution and distribute into 20 ml portions. 1 portion per 50 ml Falcon containing worm soup.
10. Prepare 4x 14 ml Falcons per 1x 50 ml Falcon with worm soup. Keep on ice.
11. Add the 20 ml bleaching solution to each 5 ml worm soup Falcon. Immediately start vortexing at full speed for 5 min.
12. Quickly distribute the worm + bleach solution into the 30ml ice-cold M9 falcons to stop the reaction. Mix briefly by tube inversion.
13. Distribute this solution into the 14 ml Falcons prepared earlier.
14. Spin embryos down @ 600 xg for 2 min.
15. Remove supernatant and fill up with fresh M9.
16. Repeat wash 2 times.
17. Pool solutions into 1x 14ml falcon.
18. Control bleaching success under stereomicroscope. Look out for:
    - Embryos present? → If not, they are either still in the worms or are completely dissolved during the bleaching step.
    - Embryos did dissolve? → Shorten bleaching time to 2-3 minutes, use more starting material for each bleaching reaction (close to 5 ml is good),
    - Worm carcasses present that contain more embryos? → Possibly repeat bleaching
    - Do embryos stick together /clump? → If yes, bleach solution attacked the shell and bleaching step should not be repeated! Otherwise considerable loss of embryos!
    - Many empty worm carcasses present? → If yes, you can filter the solution using a <40µm filter sieve.
19. Depending on bleaching success continue or filter and repeat bleaching.
20. Aliquot clean embryo soup into eppis for storage.
21. Spin top down 1000 xg in table centrifuge for 30 seconds and remove excess liquid.
22. Snap freeze embryos in liquid N2 for storage and keep at -80

Generating embryo extract:

1. Thaw embryos in 1 volume IP buffer on ice.
2. Lyse embryos via sonication (for instance by using digital cell disrupter 450-D (Branson)) to create embryo extract.
    - 450-D Sonicator settings:
        i. Pulse On: 0.5s
        ii. Pulse Off: 2.0s
        iii. Total time: 2x 10s
        iv. Amplitude: 30%
3. Depending whether you want full extract or only cytoplasmic extract, spin the extract to remove embryo debris or even larger subcellular structures that might have survived the sonication. Typically you want to centrifuge at least at 1200 xg for 10 minutes to sediement most of the debris. Longer spin times and higher speeds will also remove sub cellular structures that have survived the sonication. Test which speeds work best for your application.
4. Determine protein concentration in the supernatant by UV absorption for instance by using a NanoDrop ND-1000 Spectrophotometer.
5. This extract can be aliquoted, frozen in liquid N2 and stored at -80 for later uses.

Reagents

Immuno Precipitation (IP) Buffer (1x):

- 1x PBS
- 100 mM KCl
- 1 mM $MgCl_2$

- 1 mM EGTA
- 10 % Glycerol
- 1 % CHAPS → does not absorb at 280 nm like other detergents and thus allows measurement of protein concentration in the extract.
- Protease Inhibitors
- pH = 7.4