

Protocol for *C. elegans* cell culture and RNA isolation**Reagents needed**

- Chitinase solution (Sigma, C6137 or C7809, 1U/ml in sterile egg buffer)
- Egg buffer (118 mM NaCl, 48 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, 25 mM HEPES, pH 7.3, 340 mOsm)
- M9 buffer
- L15-10 culture media (L15 + 10% fetal bovine serum + pen/strep, adjusted to 340 mOsm with sterile sucrose)
- Poly-lysine coated coverslips or chamber slides (Nunc)

1. Isolate embryos from at least 4x10cm NA22 plates with large numbers of gravid adults.
2. Separate eggs from debris by floating embryos on 30% sucrose
3. Remove the eggs from the top of the sucrose gradient and wash twice with egg buffer
4. Incubate 50-200µl of embryos in 500µl of 0.5U - 5U /ml of chitinase in egg buffer for 45 minutes - 2 hours
5. When most of the eggshells have lysed, add 800µl of L15-10 media
6. Pellet the cells by spinning at 900xg for 3 minutes
7. Gently dissociate the cells by repeatedly pipetting using a 1ml eppendorf pipettor. Monitor dissociation by placing a drop of the suspension on a slide and examining with the 20x lens. Continue dissociation until a large number of single cells are present. There will also be some undissociated embryos, clumps of cells and hatched larvae.
8. Pellet dissociated cell suspension at 900xg for 3 minutes and resuspend in 500µl of L15-10
9. Filter cells through a 5µ filter
 - a. Using an 18g needle and 3-10 ml syringe, draw 1ml of L15-10 media into the syringe, followed by the cell suspension (take care not to mix the two!)
 - b. Attach the 5µ filter and force the solution into a sterile 1.5ml tube with medium pressure
 - c. To maximize yield, rinse the filter with 1-1.5ml of L15-10 media
 - d. Gently force this rinse into a second 1.5ml tube
10. Pellet filtered cells at 900xg for 3 minutes
11. Resuspend cells in 25-200µl of L15-10 and combine the two tubes into a single sample.
12. Determine cell density using a hemocytometer
13. Plate at a density of 10 million cells / ml on a poly-lysine coated single-well chambered coverglass.
14. Incubate cells for 24 hours at 20-25° in a humidified chamber.

FACS sorting

1. Immediately prior to sorting, remove the cell culture media and add 1ml of egg buffer to the chamber coverglass.
2. Dissociate cells from the coverglass with gentle pipetting
3. Add 2 ml of egg buffer to the slide.
4. Draw all 3ml of egg buffer + cells into a 3cc syringe and filter with a 5µ syringe filter
5. Add propidium iodide to the wild type, non-fluorescent sample at a concentration of 5µg/ml prior to sorting
6. Determine the sorting gates for damaged cells using propidium iodide stained wild type cells.
7. Use these sorting gates to isolate viable GFP or dsRed expressing cells
8. Collect cells into 100µl of Trizol for total RNA isolation.
9. Perform standard total RNA isolation using the combined Trizol/Qiagen RNAeasy method.