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Embryo stage C. elegans dissociation for FACS isolation and RNA-seq analysis

Forked from Embryo stage C. elegans dissociation for FACS isolation and RNA-seq analysis of intestine-specific cells

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We use this protocol and it's working

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Abstract

This protocol is for generating a single cell suspension suitable for isolation of germ-layer-specific cells through Fluorescence Activated Cell Sorting (FACS) from embryo stage *C. elegans*. This protocol utilizes treatment with Chitinase and Pronase E to disrupt the cuticle. Embryos are mechanically homogenized with 21G syringe needle.

Materials

Strains:
- FACS control *C. elegans* strain, i.e. N2
- FACS sorting *C. elegans* strain, i.e. JM149 *caIs71*[elt-2p::GFP::HIS-2B::unc-54 3'UTR + rol-6(su1006)]

Reagents:
L15-10 solution
- Leibovitz's L-15 Medium (Thermo 21083027)
- Fetal Bovine Serum (heat inactivated) (Thermo 10438026)
- 100X Penicillin Streptomycin solution (Thermo 15140148)
- Sucrose powder

Stock solutions for egg buffer
- 2M NaCl
- 2M KCl
- 1M CaCl2
- 1M MgCl2
- 1M HEPES pH 7.2

Enzymes
- Chitinase from Streptomyces griseus (Sigma C6137-5UN)
- Pronase E, Protease from Streptomyces griseus (Sigma P8811-1G)

Consumables:
- standard 1.5 ml tubes
- Stericup 0.2 micron filter (Fisher S2GPU05RE)
- 21 guage 1 inch needle (fisher 14-826C)
- 1 ml syringe (fisher 14-823-30)
- 35-micron nylon mesh filter caps (Stellar Scientific FSC-FLTCP)
- 5 ml sterile polypropylene round-bottom tube (STEMCELL Technologies 38057)
- Bio-Rad TC20 automated cell counting slide (Bio-rad 1450011)

Equipment:
- Fixed angle rotor centrifuge (Eppendorf 5424)
- Swinging bucket rotor refrigerated centrifuge (eppendorf 5810R)
- 15 ml tube and 1.5 ml tube adapter (eppendorf 022638704, eppendorf 022638742)
- Fluorescent microscope
- Nutating mixer
- Bio-Rad TC20 automated cell counter

Before beginning

1 Prepare reagents in advance

L15-10 Buffer: Mix 500 ml Leibovitz's L-15 Medium, 50 ml Fetal Bovine Serum (heat inactivated), 50 ul of 100x Penicillin-Streptomycin solution and 7.7 g sucrose. Filter with 0.2 micron pore filter. Store at 4ºC.

Egg Buffer: Mix 29.5 ml of 2M NaCl, 12 ml of 2M KCl, 1 ml of 1M CaCl2, 1 ml of MgCl2, 12.5 ml of 1M HEPES-NaOH pH 7.2 and 435 ml molecular grade water. Filter with 0.2 micron pore filter. Store at 4ºC.

Chitinase solution (1 U/ml): Dissolve 5 units of Chitinase from Streptomyces griseus (Sigma C6137-5UN) in 5 ml of Egg Buffer. Nutate the solution for approximately 10 minutes until dissolved. Prepare 1 ml aliquots in 1.5 ml tubes. Store aliquots at -20ºC.

Pronase E solution (15 mg/ml): Weigh 150 mg of Protease from Streptomyces griseus (Sigma P8811-1G) into a 15 ml tube. Dissolve the enzyme in 10 ml of Egg Buffer. Nutate the solution for approximately 10 minutes until dissolved. Prepare 1 ml aliquots in 1.5 ml tubes. Store aliquots at -20ºC.

2 On day of protocol:

Cool swinging bucket centrifuge to 4ºC
Thaw pronase and chitinase aliquots at room temperature
Place L15-10 and egg buffer on ice

3 Starting material:
Worm suspension in 15 ml tube (material generated from **this protocol**)
Strains: N2, fluorescent sorting strain

Perform this protocol on both strains in parallel

Note: The volumes for enzymatic treatments in this protocol require an embryo pellet less than 200 uL. If embryo pellet exceeds 200 ul, utilize 2x the embryo pellet volume.

Chitinase Treatment

4 Centrifuge embryo suspension at 2000 rcf for 1 minute in swinging bucket centrifuge

5 Aspirate and discard the supernatant

6 Transfer 10 ul of embryo pellet to 1ml of Qiazol and store at -80°C for downstream RNA analysis

7 Resuspend the embryo pellet from Step 7 in 0.5 ml egg buffer and transfer to a 1.5 ml tube containing 1 ml chitinase (1 U/ml)

8 Incubate for 20 min rotating/nutating at room temperature. During this step, cool down theswinging bucket centrifuge to 4ºC

9 Verify eggshell digestion by visualizing the chitinase treated embryos under a microscope. Early embryos should change shape, and pretzel stage embryos should release from their eggshell.

10 Pellet the embryos at 200 rcf for 5 min in fixed angle rotor centrifuge

11 Aspirate and discard the supernatant

Pronase treatment and dissociation

12 Resuspend the chitinase treated embryo pellet in 200 ul pronase (15 mg/ml) and 500 ul egg buffer

13 Attach a 21 guage 1¼ inch needle to a sterile 1 ml syringe

14 Disrupt the embryo vitelline membrane and release cells by passing the embryo suspension through the needle 90-100 times (45-50 times up and down), generating a worm slurry

15 Visually confirm embryo dissociation by viewing a 2 ul sample of worm slurry on a fluorescent microscope

16 Quench the pronase treatment by adding 800 ul of L15-10 media to worm slurry and transfer to a 5 ml tube.

17 Store the sample on ice until all strains are completed

Wash and harvest single cells

18 Wash away excess pronase from the worm slurry

18.1 Pellet the worm slurry at 500 rcf for 5 mins in swinging bucket centrifuge cooled to 4ºC

18.2 Aspirate and discard the supernatant

18.3 Resuspend the worm slurry in 1 ml of L15-10 media.

18.4 Pellet the worm slurry at 500 rcf for 5 mins in swinging bucket centrifuge cooled to 4ºC

18.5 Aspirate and discard the supernatant

18.6 Resuspend the worm slurry in 1 ml of L15-10 media.

19 Harvest the cells

19.1 Pellet undissociated embryos at 50 rcf for 30 seconds in swinging bucket centrifuge cooled to 4ºC

NOTES:
- This step will separate the dissociated cells from intact embryos
- Cells will remain in the supernatant
- Ensure your cell type of interest is not lost during this step.
- Visually confirm fluorescent cells **are present** in the supernatant.
- Visually confirm fluorescent cells **are not present** in the pellet.
- You may need to reduce the centrifuge speed and/or time if fluorescent cells are in the pellet of this step.

19.2 Aspirate 1 ml of the cell-containing supernatant. Keep the pipette away from the pelleted worm debris.

19.3 Dispense the cell suspension though a 35-micron nylon mesh filter into a 5 ml flow cytometry tube

19.4 Pellet undissociated embryos at 50 rcf for 30 seconds in swinging bucket centrifuge cooled to 4ºC

20 Perform an additional round of cell harvest for the sorting strain only (Step 21)
Total cell suspension volumes:
- Control strain = 1ml
- Sorting strain = 2ml

21 - Transfer 70 ul of cells to 1 ml of Qiazol and store at -80°C for downstream RNA analysis
- Continue to step 24
- Retain the remaining ~2ml of cells for **FACS**

Measure approximate cell concentration

22 Load 10 ul of cell suspension to a **Bio-Rad** TC20 automated cell counting slide

23 This protocol should yield between 2×10^6 to 4×10^6 total cells

24 Dilute the sample to $1 \times 10^6 \frac{\text{cells}}{\text{ml}}$ if above this concentration with L15-10

25 Microscopically confirm fluorescent cells are present in the cell suspension

26 Move on to **FACS protocol**