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

In 1 collection

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ABSTRACT

This protocol is for generating a single cell suspension suitable for isolation of intestine-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.

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COLLECTIONS

**Protocol collection: Dissociation and FACS isolation of embryonic and post-embryonic C. elegans intestine cells for RNA-seq analysis**

KEYWORDS

C. elegans, FACS, single cell suspension, cell dissociaiton, embryo stage, intestine



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MATERIALS TEXT

**Strains:**
- FACS control *C. elegans* strain, i.e. N2
- FACS sorting *C. elegans* strain, i.e. JM149 *cals71*[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



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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 Resuspend the embryo pellet in 1 ml of M9 and transfer to a 1.5 ml tube.

6 Pellet the embryos at 2000 rcf for 1 minute in a centrifuge

7 Aspirate and discard the supernatant

8 ⚠



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

9 Resuspend the embryo pellet from Step 7 in 0.5 ml egg buffer and 1 ml chitinase (1 U/ml)

10 Incubate for 20 min rotating/nutating at room temperature

11 ⚠

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.

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

13 Aspirate and discard the supernatant

## Pronase treatment and dissociation

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

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

16 Disrupt the embryo vitelline membrane and release cells by passing the embryo suspension through the needle 100 times, generating a worm slurry

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

18 Quench the pronase treatment by adding 800 ul of L15-10 media to worm slurry



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19 Store the sample on ice until all strains are completed

## Wash and harvest single cells

20 Wash away excess pronase from the worm slurry

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

20.2 Aspirate and discard the supernatant

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

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

20.5 Aspirate and discard the supernatant

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

21 Harvest the cells

21.1 ⚠



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Pellet undissociated embryos at 100 rcf for 1 minute 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.

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

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

21.4 Pellet undissociated embryos at 100 rcf for 1 minute in swinging bucket centrifuge cooled to 4ºC

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

23 - 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

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

25 This protocol should yield between $2 \times 10^6$ to $4 \times 10^6$ total cells

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



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27 Microscopically confirm fluorescent cells are present in the cell suspension

28 Move on to FACS protocol



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