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 We use this protocol and it's working

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## 🌐 L1 stage *C. elegans* dissociation for FACS isolation and RNA-seq analysis of intestine-specific cells V.2

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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 L1 stage *C. elegans*. This protocol utilizes treatment with SDS-DTT solution and Pronase E to disrupt the cuticle. Worms are mechanically homogenized with a dounce homogenizer.

**Keywords:** *C. elegans*, intestine, FACS, single cell suspension, cell dissociation, L1 stage

## MATERIALS

### Strains:

- FACS control *C. elegans* strain, i.e. N2
- FACS sorting *C. elegans* strain, i.e. JM149 *cal-51*[*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 CaCl<sub>2</sub>
- 1M MgCl<sub>2</sub>
- 1M HEPES pH 7.2

Enzymes

- Pronase E, Protease from *Streptomyces griseus* (Sigma P8811-1G)

SDS-DTT

- 1M DTT
- 10% SDS
- 1M HEPES pH8
- 60% sucrose

### Consumables:

- standard 1.5 ml tubes
- Stericup 0.2 micron filter (Fisher S2GPU05RE)
- 20-micron mesh filter (Fisher Scientific NC1004201)
- 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
- 2ml Dounce homogenizer with pestle A (Sigma-Aldrich D8938)

## 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 µl of 100x Penicillin-Streptomycin solution and 7.7 g sucrose. Filter with 0.2 micron pore filter. Store at 4°C.

**Egg Buffer:** Mix 29.5 ml of 2M NaCl, 12 ml of 2M KCl, 1 ml of 1M CaCl<sub>2</sub>, 1 ml of MgCl<sub>2</sub>, 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 4°C.

**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 -20°C.

#### **SDS-DTT solution:**

- To ensure treatment efficacy, the SDS-DTT solution is prepared fresh on the day of the experiment. Additionally, several aliquots of DTT powder in 1.5 ml tubes are prepared in advance and stored at -20°C.
- To prepare the SDS-DTT solution, first resuspend 154 mg of DTT powder with 1 mL of Egg Buffer on the day of the experiment. Then, combine the following reagents in a 15 ml tube (final concentrations in parenthesis): 100 µL of 1M HEPES pH8 (20 mM), 125 µL of 10% SDS (0.25%), 1 mL of 1M DTT (200 mM), 250 µL of 60% sucrose (3%), and 3.525 mL of molecular grade water for a final volume of 5 mL.
- The SDS-DTT solution is one of the most important reagents to ensure successful dissociation of post-embryonic worms. To check that the SDS-DTT solution is working properly, incubate worms in freshly prepared SDS-DTT solution under a dissection microscope. When prepared properly, worms should dissolve within ~10 minutes of observation.

### 2 On day of protocol:

Cool swinging bucket centrifuge to 4°C

Thaw pronase aliquots at room temperature

Place L15-10 and egg buffer on ice

Wash and sterilize dounce homogenizer and pestle A (1ml H<sub>2</sub>O, 1ml 70% EtOH)

### 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 chemical and enzymatic treatments in this protocol require an L1 pellet less than 200 uL. If L1 pellet exceeds 200 ul, utilize 2x the L1 pellet volume.

## Harvest L1 Worms

- 4 Harvest the synchronized L1 worms by washing the plates with fresh M9.
- 5 Pass the harvested L1 suspension through a 20 micron filter. This will filter any contaminating debris (agar chunks, partially bleached worm chunks) and any unhatched or dead embryos.
- 6 Pellet the worms in a 15 ml tube for 1 min at 2,000 rcf. Discard the supernatant and repeat M9 washes until the supernatant is free of visible E. coli.

## SDS-DTT Treatment

- 7 Once the worm suspension is free of E. coli, centrifuge worm suspension at 2,000 rcf for 1 minute
- 8 Resuspend the worm pellet in 1 ml of M9 and transfer to a 1.5 ml tube.
- 9 Pellet the worms again at 2,000 rcf for 1 minute.
- 10 Transfer 10 ul of L1 worm pellet to 1ml of Qiazol and store at -80°C for downstream RNA analysis

- 11 Resuspend the worm pellet in 200 ul of fresh SDS-DTT solution
- 12 Incubate for 2 min room temperature to digest the cuticle  
**NOTE:**
  - Worms should look ruffled, and pharynx should change from thin and elongated to short and round
  - Incubation exceeding 2 min will damage the sample
- 13 Quench reaction by adding 800 ul of ice cold egg buffer
- 14 Pellet worms at 13,000 rcf for 15 seconds at 4°C
- 15 Decant the supernatant and replace with 1 ml of ice-cold egg buffer
- 16 Repeat the egg buffer wash for a total of 5 washes

## Pronase E Treatment

- 17 Resuspend the SDS-DTT treated worms in 200 ul of 15 mg/ml Pronase E
- 18 Incubate for 30 minutes at room temperature nutating to digest the cuticle

- 19 Quench the Pronase E treatment by adding 1 ml of L15-10
- 20 Pellet the worms at 13,000 rcf for 15 seconds at 4°C
- 21 Decant the supernatant and resuspend the worms in 1 ml of L15-10
- 22 Pellet the worms at 13,000 rcf for 15 seconds at 4°C
- 23 Resuspend the worms in 1ml of L15-10

## Cell dissociation

- 24 Transfer the worm suspension to a 2 ml glass dounce homogenizer
- 25 Perform 100 strokes with dounce pestle A to generate a worm slurry
- 26 Visually confirm worm dissociation by viewing a 2 ul sample of worm slurry on a fluorescent

microscope

## 27 Harvest the cells

### 27.1 Transfer worm slurry to 1 ml centrifuge tube

### 27.2 Pellet undissociated worms at 100 rcf for 1 minute at 4°C in swinging bucket centrifuge



#### NOTES:

- This step will separate the dissociated cells from intact worms
- Cells will remain in the supernatant
- Intact and partially dissociated worms will remain in the pellet
- 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.

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

### 27.4 Dispense the cell suspension through a 35-micron nylon mesh filter into a 5 ml flow cytometry tube

## 28 Resuspend remaining worm slurry in 1ml of L15-10

## 29 For the sorting strain, perform an additional round of dissociation and cell harvest (Steps 24-28). You will perform a total of two to three homogenization cycles, until few intact worms remain.

Total cell suspension volumes:

- Control strain = 1ml
- Sorting strain = 2-3ml

- 30**
- Transfer 70 ul of cells to 1 ml of Qiazol and store at -80°C for downstream RNA analysis
  - Continue to step 31
  - Retain the remaining ~2ml of cells for [FACS](#)

## Measure approximate cell concentration

- 31** Load 10 ul of cell suspension to a Bio-Rad TC20 automated cell counting slide. Also can use a hemocytometer.
- 32** This protocol should yield between  $2 \times 10^6$  to  $4 \times 10^6$  total cells
- 33** Dilute the sample to  $1 \times 10^6 \frac{\text{cells}}{\text{ml}}$  if above this concentration with L15-10
- 34** Microscopically confirm fluorescent cells are present in the cell counting slide
- 35** If the total cell yield is less than  $1 \times 10^6$  cells and many intact worms remain, repeat the SDS-DTT, Pronase E and dounce homogenization steps
- 36** Move on to [FACS protocol](#)



