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FACS isolation of intestine-specific C. elegans cells

In 1 collection

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ABSTRACT

This protocol describes the steps necessary for FACS isolation of *C. elegans* intestine cells. Section 1 contains general guidelines and considerations to be made before performing a FACS experiment. Sections 2-4 detail how to prepare cells for a specific developmental stage. Sections 5-7 detail how to prepare the FACS instrument for sorting and evaluating the purity of isolated cells. Section 8 describes how to handle cells once they have been isolated.

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COLLECTIONS

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

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PARENT PROTOCOLS

Part of collection

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

MATERIALS TEXT

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)]

Consumables:
- DRAQ5 stain (Thermo 62251)
- ReadyFlow Propidium Iodide (Invitrogen R37169)
- 5 ml sterile polypropylene round-bottom tube (STEMCELL Technologies 38057)
- Qiagen miRNEasy Micro kit (Qiagen 217084)

Equipment:
- BD FACSAria III Cell sorter

Before beginning

1 The starting material required to perform this procedure was generated with either of the following protocols:
Embryo Stage Dissociation
L1 Stage Dissociation
L3 Stage Dissociation

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2 Ensure your cells do not exceed a concentration of $1 \times 10^6 \frac{\text{cells}}{\text{ml}}$. Cell suspensions exceeding this concentration are challenging to analyze on the FACS instrument for cells of this size and may increase the probability of doublets/hitchhiking cells.

3 This protocol utilizes dye combinations determined optimal for the corresponding stage and cell suspension concentration. Alternative dye combinations can be used, but ensure that the dyes used do not spectrally overlap. Consult resources such as FluoroFinder. When using new dyes, perform a staining index experiment to determine the optimal stain concentration to use (additional information here).

Dye rationale:
Embryo stage - Stain with viability dye Propidium Iodide to separate live cells (PI-) from dead cells (PI+)
L1 stage - Viability dyes are not used, as intestine cells preferentially take up viability dyes and will confound sorting
L3 stage - Similar to L1, viability dyes are not used. L3 cell preps have a high degree of debris, so a cell permeable nucleic acid dye such as DRAQ5 is used to distinguish cells (DRAQ5+) from debris (DRAQ5-).

4 When first performing this assay, aim to microscopically visualize samples isolated through FACS as a primary endpoint for assay success. Intestine samples should be GFP+, resemble intestine cell morphology, and contain few to no GFP- cells. Alternatively, non-intestine samples should be GFP- and contain few to no GFP+ cells. Once confident in the sorting procedure, samples do not need to be microscopically visualized for each experiment to preserve material for RNA-seq analysis.

5 The following steps describe how to prepare samples necessary for FACS isolation of intestine cells. If using embryo samples, follow Steps 6-9 for cell preparation. If using L1 samples, follow Steps 10-11. If using L3 samples, follow Steps 12-15. Once cells are prepared, proceed to step 18 for FACS.

Embryo stage cell prep

6 Divide samples into flow tubes with the following volumes and labels

A	B	C	D
Sample type	**Worm strain**	**Dye**	**Cell volume (ml)**
Unstained	N2	None	0.5
GFP single	GFP strain	None	0.5
PI single	N2	Propidium Iodide	0.5
Sorting sample	GFP strain	Propidium Iodide	2-4

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7 To the appropriate tubes indicated in Step 6, Add two drops of ReadyFlow Propidium Iodide per $1 \times 10^6 \frac{\text{cells}}{\text{ml}}$

8 Incubate for 15 mins on ice protected from light

9 Proceed to FACS (Step 18)

L1 stage cell prep

10 Divide samples into flow tubes with the following volumes and labels

A	B	C	D
Sample type	**Worm strain**	**Dye**	**Cell volume (ml)**
Unstained	N2	None	1
Sorting sample	GFP strain	None	2-4

11 Proceed to FACS (Step 18)

L3 stage cell prep

12 Divide samples into flow tubes with the following volumes and labels

For L3 stage experiments:

A	B	C	D
Sample type	**Worm strain**	**Dye**	**Cell volume (ml)**
Unstained	N2	None	0.5
GFP single	GFP strain	None	0.5
DRAQ5 single	N2	DRAQ5	0.5
Sorting sample	GFP strain	DRAQ5	2-4

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13 To the appropriate tubes indicated in Step 12, add 1 ul of DRAQ5 per ml of cell volume

14 Incubate for 30 minutes on ice protected from light

15 Wash cells to remove excess dye. Pellet cells by centrifuging for 5 minutes at 530 rcf in 4C swinging bucket centrifuge.

16 Resuspend cells in L15-10 equal to their starting volume

17 Proceed to FACS (Step 18)

FACS Setup

18 The following protocol steps were developed on a BD FACSAria III instrument. The settings and recommendations detailed here may be adjusted as necessary.

Setup the instrument:
- Set laser gain and area scaling factor on the FACS instrument to ensure that collected data falls within the plotting area.
- Set plots to log-log axis

NOTE:
C. elegans cells are smaller than material typically run on a FACS instrument. Compared to mammalian cell lines, C. elegans cells require a lower area scaling factor and lower laser voltages. Additionally, set plot axes to log-log transformed.

19 Run the unstained control cell samples through the FACS instrument. Adjust laser voltages and area scaling factors to ensure that collected data (i.e. SSC and FSC) falls within the plotting area. Once values are set properly, record data from ~10,000 events

20 Run the all appropriate single stain control samples through the FACS instrument and record data from ~10,000 events. Set gates based on single stain controls.

21 Run the sorting sample through the FACS instrument and record data from ~10,000 events

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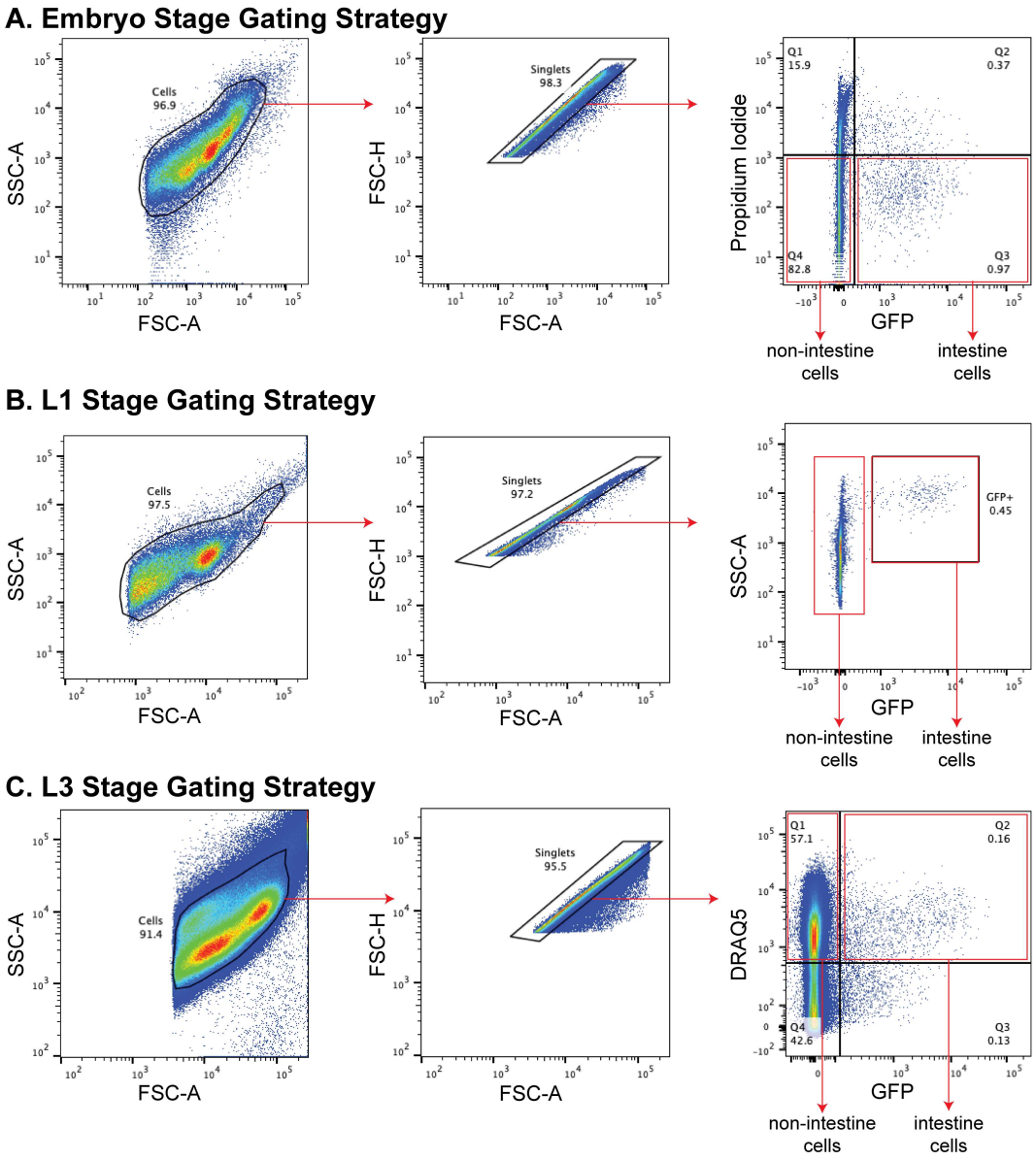
22 Verify that gates are set appropriately for the sorting sample and adjust gates as needed

23 Blow are example gating strategies utilized for intestine isolation. Simultaneously collect cells within the "intestine cells" and "non-intestine cells" gates.



Example gating strategy for isolating intestine cells from embryo stage (A), L1 stage (B), and L3 stage (D) dissociated cell preps.

Sort cells

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24 Once gates and gating strategy are established, prepare two flow tubes to collect the sorted cells by aliquoting 0.2ml of L15-10 into them. Place the collection tubes into the tube holder inside the FACS instrument.

25 Prepare the FACS instrument for a simultaneous two-way collection of cells from the "non-intestine cells" gate and "intestine cells" gate outlined in Step 23

26 Collect $1x10^6$ cells from the non-intestine cells gate. This should yield a total volume of 3.5-4ml of cell suspension. Store sorted cells on ice.

27 Collect cells from the "intestine cells" gate continuously until there is no more sorting sample remaining. This should yield between 50,000 to 300,000 cells in a total volume of 0.2-0.5ml. Store sorted cells on ice.

Post-sort purity analysis

28 Once sorting is complete, perform a post-sort purity analysis. This will determine if the FACS run was successful.

29 ⚠

The microfluidic line must be cleaned after sorting and before measuring post-sort purity for each sample. Clean the line by running a sample of fresh 10% bleach for ~5 mins. Follow this by running a sample of filtered H20 ~2mins. The FACS instrument should record fewer than 1 event/sec.

30 Run the intestine cell sample back through the instrument. The intestine cell post-sort purity should be 80-90%.

31 Clean the microfluidic line following the procedure outlined in Step 29. Run the non-intestine cell sample back through the instrument. The non-intestine cell post-sort purity should be 100%.

Sample preparation for RNA extraction

32 Prepare the cells for downstream RNA analysis: Pellet intestine and non-intestine cells at 10,000 rcf for 5 mins in 4C cooled centrifuge. There should be a small but visible pellet in the intestine cell population.

33 Decant supernatant and resuspend cells in 1 ml Qiazol. Store at -80°C until ready for RNA

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isolation and quantification.

34 We utilize the Qiagen miRNEasy kit for RNA isolation. Quantification is performed with High Sensitivity RNA kits from Qubit and Tapestation. By following the protocols in this collection, expect approximately 10 ng total RNA for intestine cell samples (50-300,000 cells) and >100 ng total RNA for non-intestine cell samples ($1x10^6$ cells).

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