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Tissues dissociation in Daphnia

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[1]Mission Bio

Indira Krishnan: This protocol was primarily developed by Indira Krishnan while at Harvard Medical School.

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Abstract

Tissues dissociation in Daphnia for SC library preparation

Guidelines

Fluorescence (Ex/Em: 488 nm / 650 nm) indicative of dead cells. Ten ul of the original cell suspension was mixed with 1 ul of 1 mM Hoechst 33342 and cells counted under microscope in the DAPI channel to determine the number of cells/mL. Cells were then diluted to approximately 90,000 cells / mL in the wash buffer with 15% (v/v) of Optiprep before loading for transcriptome barcoding of single cells on the inDrop platform.

Materials

- Tetracycline HCl
- Streptomycin sulfate
- Ampicillin sodium salt
- ADaM medium
- HBSS buffer
- ThermoFisher
- Collagenase
- Hyaluronidase
- SigmaAldrich
- Sodium phosphate buffer
- HEPES
- CaCl2
- BSA
- Microscalpel
- Calcein-AM
- Ethidium homodimer III
- Promokine
- Moxi Go II flow cytometer
- Hoechst 33342
- Optiprep

Before start

Prior to tissue dissociation, Daphnia were maintained for 3 days in antibiotics solution that contained 50mg/L of each of tetracycline HCl, streptomycin sulfate, and ampicillin sodium salt in 0.2 um-filtered ADaM medium, with the solution replaced daily.

Tissues dissociation in Daphnia

1 Five adult Daphnia (approx. 10 mg wet weight) were first placed in a beaker containing HBSS buffer without Ca^{2+} and Mg^{2+}; 5:3 mOsm/L (ThermoFisher) on ice for 20 to 30 min to reduce tissues concentration of bivalent cations.

2 Daphnia were transferred into enzyme mixture solution containing 8 mg/mL collagenase and 2.8 mg/mL (2000 U/mL) hyaluronidase (both SigmaAldrich) in HBSS dissociation buffer with 20mM sodium phosphate buffer, 25mM HEPES, 0.5 mM CaCl2 and 0.1 mg/mL BSA added; pH 7.2).

3 Eggs or embryos were removed from the brood chamber and Daphnia were cut transversely into three pieces with a microscalpel while in the enzyme mixture, with the rami of the antennae II removed (to prevent serrated setae present on the rami from clogging the microfluidics equipment).

4 The samples were then placed in a shaker at 50 rpm, at 30 °C for 1 h. At the end of the incubation, tubes were placed on ice until big debris settled to the bottom and the cell suspension was passed through stacked 70 um 26 30 um filters (pre-wetted with the dissociation buffer), and rinsed with 3 mL of the same buffer.

5 The cell suspension was spun at 500 x g for 25 min at 4 °C. Supernatant was removed and the pellet was washed three times with the wash buffer (HBSS w/o Ca^{2+} and Mg^{2+}, with 25mM HEPES added, pH 7.2) and suspended in 2 ml of the same buffer.

6 Cell viability was tested by Calcein-AM/Ethidium homodimer III assay (Promokine). Briefly, working solutions of Calcein AM (400 uM) and Ethidium homodimer III (100 uM) were freshly prepared from 4 mM and 2 mM stocks, respectively, in the wash buffer. Four uL of each of the working solutions were added to 200 ul of cell suspension to a final concentration of 8 uM (Calcein AM) and 2 uM (EthD-III) and the mix was incubated in the dark at room temperature for 30 min.

7 Controls without Calcein AM and EthD-III were included for each assay. Following the incubation, the cells were pelleted by centrifugation at 200 x g for 10 min and washed twice the wash buffer. The cells were then suspended in 100 ul of the same buffer and analyzed by flow cytometry on Moxi Go II flow cytometer, with calcein fluorescence (Ex/Em: 488 nm / 525 nm) indicative of live cells, and EthD-III fluorescence (Ex/Em: 488 nm / 650 nm) indicative of dead cells.

8 Ten ul of the original cell suspension was mixed with 1 ul of 1 mM Hoechst 33342 and cells counted under microscope in the DAPI channel to determine the number of cells/mL. Cells were then diluted to approximately 90,000 cells / mL in the wash buffer with 15% (v/v) of Optiprep before loading for transcriptome barcoding of single cells on the inDrop platform.