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Stony Coral Single Cell Isolation

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We use this protocol and it's working

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Abstract

This protocol was optimized in the Davies lab at Boston University for use with the facultatively symbiotic stony coral *Oculina arbuscula*. The protocol is designed to isolate live cells for downstream use in 10X single-cell RNA-sequencing.

Materials

Reagents

Sterile DI Water
0.2 micron filter-sterilized Calcium-Free Seawater (CfFSW)

Fetal Bovine Serum (FBS)
10X PBS
1X PBS
1M HEPES

Protector RNase Inibitor (Sigma Aldrich 3335399001)
0.4% Trypan Blue Solution (filtered through 0.2 micron filter to remove aggregates)

RNase Zap or equivalent

Materials

Heat-bath or hot-plate capable of 56°C
6-well plate
Sterile 200µl pipette tips
Forceps/tweezers
Sterile 1.5 ml tubes
Sterile 15 ml conical tubes
Sterile 70 micron cell strainers (e.g., Fisher Scientific 08-771-2)
Sterile 40 micron cell strainers (e.g., Fisher Scientific 08-771-1)
Microcentrifuge capable of 4°C
Hemocytometer
Light Microscope

Safety warnings

1. As much as possible, all steps should be performed on ice
2. Do not vortex cell suspension at any point during the protocol
3. Limit bubbles in cell suspension
4. Cells should be processed promptly following dissociation to maintain viability (viability decreases within an hour following isolation)

1 Heat inactivate FBS for 30 minutes at 56°C

2 Sterilize tools (i.e., forceps, pipettes, etc.) and bench space with RNAse Zap

3 Prepare cell media[1] and keep on ice

3.1 To make 20 ml of cell media:
- 6.6 ml 10X PBS (final concentration of 3.3X)
- 0.4 ml heat-inactivated FBS (final concentration of 2% by volume)
- 0.4 ml 1M HEPES (final concentration of 20mM)
- Fill to 20 ml with sterile DI H_2O

4 Add 10 mls of CafFSW[2] to two wells of a 6-well plate, and add 10 mls of cell media to a third well. Keep plate on ice.

5 Obtain live coral frag (this protocol has been optimized for frags of symbiotic or aposymbiotic branching coral ~1.5 cm in length and ~1 cm in width) and place in first well of CafFSW

6 Incubate for 1 minute (on ice)

7 With sterilized forceps, move frag to second well of CafFSW, and incubate for 1 minute (on ice)

8 With sterilized forceps, move frag to well containing cell media. Using forceps and sterile 200 µl pipette tips, carefully scrape all tissue from the coral skeleton and from within the polyps[3]. This step should last no more than 10 minutes. If necessary, this step can be performed on the bench, not on ice.

TIP: Dig the pipette tip or forcep tip into a polyp and make small circles to extract cells from within the polyp

9 Filter full cell suspension through one 70 micron cell strainer into a 15 ml conical tube.

NOTE: for filtration steps, pipette 1 ml aliquots through strainer by holding tip against mesh, depressing the plunger slowly and assuring all liquid passes through the strainer. Keep plunger depressed while removing tip from mesh. Cell suspension may be mucus-y at this point, so some volume (and cells) will inevitably be lost.

10 Filter 70-micron-filtered suspension through one 40 micron cell strainer into a new 15 ml conical tube

11 Filter suspension through a second 40 micron cell strainer into a third 15 ml conical tube

12 Centrifuge 1 ml aliquot(s) of filtered cell suspension at 300xg for 10 minutes at 4°C

13 Discard supernatent and resuspend pellet in 100 µl of cell media with 0.2 U/L Protector RNase Inhibitor

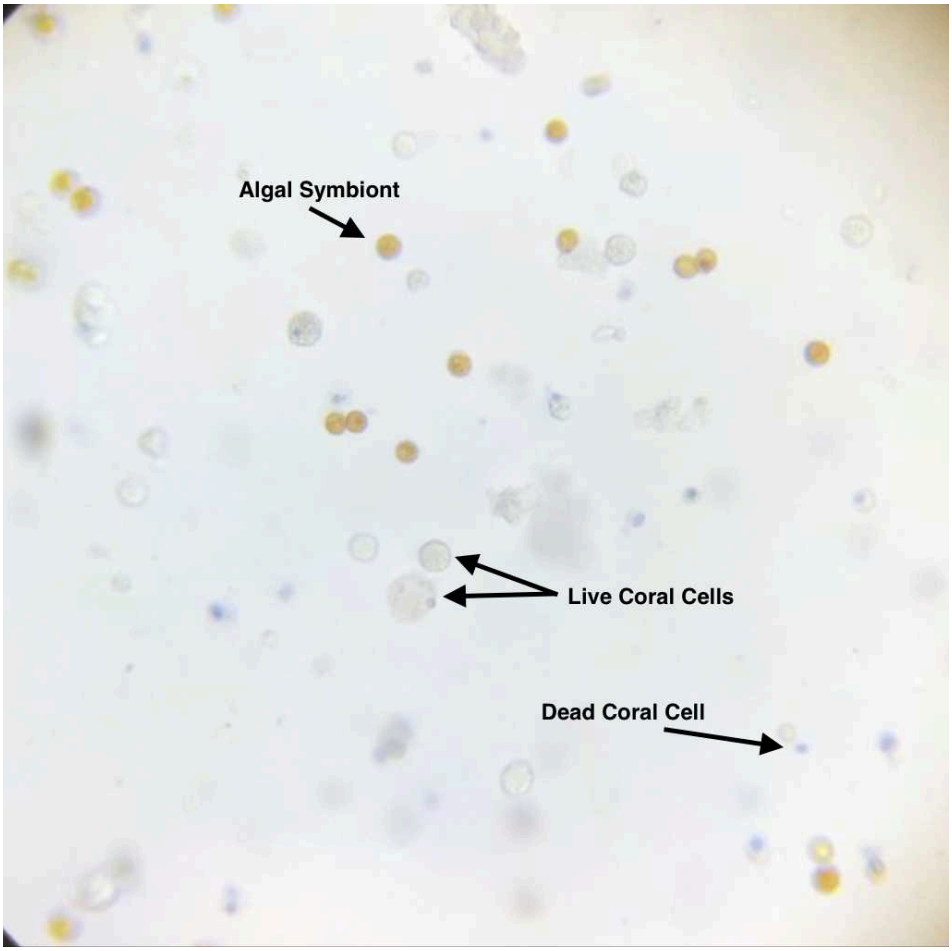
13.1 Right before use, add 0.5 µl of stock RNase Inhibitor (at 40 U/µl) per 100 µl cell media

14 Check cell viability and perform cell counts (using hemocytometer)

14.1 Combine 2.5 µl of 0.4% Trypan Blue with 12.5 µl of 1X PBS and 10 µl of final cell resuspension (for final concentration of 0.04% Trypan)

14.2 For 10X, cell viability should be over 90% (dead cells are completely permeated with blue dye)

14.3 Symbiotic cells (algal cells) should be counted in cell count and viability calculations



Example of cell suspension from symbiotic *Oculina arbuscula* with 0.04% Trypan Blue visualized at 40X magnification

15 Dilute cells to desired concentration in cell media with RNase Inhibitor (see step 13.1 for concentration)

15.1 For 10X at the Boston University Single Cell Core, cells were provided at concentrations of 700-1,200 cells/µl

Protocol references

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