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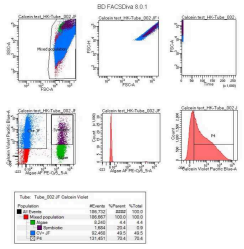
Cassiopea xamachana Cellular Dissociation



Forked from a private protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is to optimized to dissociate and fix Cassiopea xamachana cells for cell sorting and scRNA-seq.
The dissociation by itself results in 53-55% of viable cells.
Cells cannot be sorted without fixative, unless your machine can sort a seawater solution. Any other solution will lyse cells.

Guidelines

Make sure to work in an RNAse free-environment when able to. Use RNAse-ZAP or work in a UV sterilized hood if possible.

The tissue should be less than 1 cm long

Materials

- Sterile razors
- Sterile forceps
- Ice
- Wide-bore pipette tips, or cut 1000 uL pipette tips
- 15 mL tube for digestion buffer
- 2 petri dishes to wash and incubate tissue in Ca^{2+} and Mg^{2+} free seawater
- 2 70-um filters
- 2 mL microcentrifuge tubes
- pipettes

Equipment:
microcentrifuge
rocking plate

Reagents:
NaCl
KCl
$NaSO_4$
$NaHCO_3$
Dispase II
Liberase
L-cysteine
PBS
BSA
DNase/RNase-free distilled water
methanol
glacial acetic acid
glycerol
RNAse Inhibitor

Troubleshooting

Before start

Treat reagents and materials with UV-light for ~15 mins before beginning protocol.

Set 15 mL and microcentrifuge to 4° C.

Prepare Reagents:

Ca^{2+} Mg^{2+} free seawater (Roger et al. 2021)
To 1 L Distilled Water add:
- 23 g NaCl
- 0.763 g KCl
- 3 g NaSO4
- 0.25 g NaHCO3

- Dissociation Mix:
To Ca^{2+} Mg^{2+} free ASW add:
- 3.6 mg/mL Dispase II
- 0.25 mg/mL of Liberase
- 4% L-cysteine

- 1x PBS 0.5% bovine serum albumin (BSA)
- Add 0.25 g to 50 mL 1x PBS.

- Fresh ACME Solution
- 13:3:3:2 ratio of DNase/RNase-free distilled water, methanol, glacial acetic acid, and glycerol
-Prep about 15 mL, FRESH, per sample each time

- RNAse Inhibitor

Dissociation

1h

1 Cut the jellyfish tissue with a sterile razor to encourage permeability of reagents. 2m

2 Gently wash the jellyfish tissue in 10 mL Ca-Mg-Free SW for 00:01:00 then transfer to fresh 10 mL Ca-Mg-Free SW and let incubate at Room temperature for 00:02:30 . 3m 30s

3 Using sterilized forceps, place the jelly tissue into a clean 15 mL tube then add 1 mL dissociation mix on top of the jelly, or enough to submerge the tissue. 30s

4 Incubate the tissue on a rocker for 00:30:00 at room temperature. 30m

5 Pipette up and down using a wide-bore tip 10 times. 5m

6 Repeat steps 4 and 5. 35m

7 Add 80 µL fetal bovine serum to the cell suspension to create a 8% FBS solution to halt enzyme digestion. 2m

8 After the incubation period, filter the sample through a 70 µm filter Keep sample On ice moving forward. 2m

9 Resuspend in 1000 µL Ca-Mg-free SW . Gently pipette up and down 10 times with a wide-bore tip to dissociate clumps. 2m

Staining and Fixation

10 Add [M] 1 micromolar (µM) Calcein Violet 450 AM to cells. Incubate in the dark for 00:30:00 . 30m

11 After the incubation period, filter the sample through a 70 µm filter 5m

12 Resuspend in 500 µL ACME . Incubate for 00:30:00 . 35m

13 Centrifuge 350 x g, 4°C, 00:07:00 then carefully discard the supernatant. 7m

14 Re-suspend the pellet in 800 µL 1x PBS w/ 1% BSA using gentle pipetting with a wide-bore pipette then add 1 µL RNA Inhibitor . 1m

FACS & Cryopreservation

15 Pre-chill chambers of FACS Machine to 4 °C 5m

16 Sort at the slowest rate (High-purity) with less than 50 PSI at 4 °C . Gate for Calcein Violet (450 nm) and chlorophyll autofluorescence (650-700 nm) for viable jelly cells and symbiont cells. 2h

17 After sorting, cryopreserve by adding [M] 10 % volume DMSO and 1 µL RNA Inhbitor and immediately putting the sample at -80 °C . 1m

Thaw and Sample Submission 38m

18 Thaw the sample On ice 30m

19 Centrifuge 350 x g, 4°C, 00:07:00 then carefully remove the supernatant. 7m

20 Re-suspend the cells in 1 mL 3.3x PBS w/ 1% BSA then add 1 µL RNA Inhbitor . 1m

21 Submit to sequencing center On ice .

Protocol references

https://github.com/thesyntheticcoral/SynCoral_Protocols/tree/master/Culturing

Roger LM, Reich HG, Lawrence E, Li S, Vizgaudis W, Brenner N, et al. (2021) Applying model approaches in non-model systems: A review and case study on coral cell culture. PLoS ONE 16(4): e0248953. https://doi.org/10.1371/journal.pone.0248953