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Parhyale hawaiiensis embryo single cell dissociation

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Protocol status: In development
We are still developing and optimizing this protocol

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PROTOCOL integer ID:
19481

Work a 4C for the whole protocol.

1 For the whole protocol, work at 4C, and place all solutions on ice.

Prepare material

- 2
- 12 well plate
 - Tungsten needles
 - Parafilm, cut into squares a little bigger than the diameter of the well.
 - Tape
 - Elmer's paste or silicon seal
 -

Coat all gear with BSA

- 3
1. Prepare 1% BSA solution in ddH₂O
 2. Coat 1 or more wells from 12 well plate
 3. Coat pasteur pipettes for embryo transfers
 4. If using syringes for loading coat tubing and syringes

Prepare dissociation buffer and PBS Optiprep

- 4 Prepare dissociation buffer, for 40ml in ddH₂O:

	quantity	unit
Isethionic acid sodium	600	mg
Sodium pyrophosphate	360	mg
CAPS	88	mg
ddH ₂ O	40	ml
Total	40	ml

Isethionic acid sodium salt: Sigma-Aldrich 220078-25G

Sodium pyrophosphate tetrabasic decahydrate Sigma-Aldrich S6422-100G

CAPS Sigma-Aldrich C2632-25G

Prepare 2ml PBS optiprep solutions, all volumes in ul

	5%	10%	20%	30%
10x PBS	200	200	200	200

ddH ₂ O	1700	1600	1400	1200
Optiprep	100	200	400	600

For 2ml final volume of 1xPBS. All volumes in ul.

Collect embryos

- 5 This protocol was tested on embryos at 12hpf to 72hpf.
From the collected females, harvest embryos in the pouch and place them in FASW.

Prepare the dissociation well

- 6
 - Remove the 1% BSA from the wells of the 12 well plate and wash with dissociation buffer once. The fill halfway with the dissociation buffer.
 - On the lid, locate the position of that well and place a ring of elmer's paste that will serve as seal.
 - Place tape on the edges of the lid to allow for it to stay in place once closed.

Remove Egg Shell

- 7
 - Place 30 embryos on a Sylgard plate or a 2% agar in FASW plate in a small drop.
 - Aspirate as much as possible of the FASW
 - Wash with a drop of dissociation buffer. Repeat until no precipitate is visible.
 - Using the tungsten needles, remove the egg shell around the embryos. Make sure to place the egg shells away from the embryos as they will prevent the correct dissociation.
 - Once all embryos have been separated from the egg shell, transfert them into the well filled with dissociation buffer.

Prepare the well for dissociation

- 8
 - Fill the well with the embryos all the way with dissociation buffer until a meniscus is visible.
 - Place the parafilm square on the meniscus. **THERE MUSN'T BE ANY BUBBLES TRAPPED.**
 - Close the lid, sealing the parafilm in place thanks to the paste, and fix it with the tape around.

Dissociate the cells

- 9
- Place the cells on a vortex at max speed for 25 minutes
 - 00:25:00

Clean the dissociation buffer

- 10
- Remove the plate from the vortex and let the cells settle for 5 minutes
 - Open the lid and assess that the dissociation worked.
 - Aspirate 1ml of liquid at the top of the well
 - Then fill from the bottom with 400 ul of 5% Optiprep PBS
 - Then 200ul of 10% optiprep
 - Then 100ul of 20% optiprep
 - And finally 300ul of 30% optiprep

Centrifuge to concentrate the cells at the 20% mark

- 11
- Centrifuge the plate at 2500 RPM for 3-5 minutes to concentrate the cells at the margin.

Collect the cells

- 12
- Using the BSA coated syringe, aspirate the cells collected at the 20% optipre band.