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Parhyale hawaiensis embryo single cell dissociation

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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
ddH2O	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
ddH2O	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.