Dechorionation of sea bass embryos and cleaning of chorion constituents

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Overview: This protocol describes a method used to extract sea bass embryos from eggs, and clean them from other egg constituents, such as proteins and oils which can interfere with downstream library preparation for ATAC-seq and ChIP-seq.

Consumables:

- Sterile 50 ml plastic tubes
- Watchmaker forceps
- 1.5ml Eppendorf tubes
- Pronase 1mg/ml in seabass-osmolar PBS 1X, (stock: 30 mg/ml)
- Seabass-osmolar PBS 1X (add 1100 µl of NaCl 2 M to 50 ml of PBS)
- Seabass-osmolar DMEM
- Seabass-osmolar FBS (heat inactivated)
- proteinase inhibitor cocktail tablet (Complete PIC tablet, EDTA free; Roche)
- 15ml Falcon tubes
- Rotator system (HulaMixer™ Sample Mixer)
- Dissection microscope
- 70 µm filters
- 6-well cell culture plates (and/or Petri dish)
- Single concave microscope slides
- Cooled incubator
- Vortex
- Fixed angle centrifuge
- Crushed ice

Seabass osmolar Resuspension buffer (RSB)
5 ml FBS
5 ml DMEM
1 proteinase inhibitor cocktail tablet (Complete PIC tablet, EDTA free; Roche)
105 μl NaCl 2M

Seabass osmolar Cryopreservant solution
4 ml FBS
5 ml DMEM
1 DMSO
91 μl NaCl 2M

2.1 Place required number of eggs in a sterile 50ml plastic tubes with 6ml of pronase 1mg/ml in seabass-osmolar PBS 1X.

2.2 Rotate for 30 min at 20RPM at 20°C in a cooled incubator.

2.3 Eliminate pronase solution and add 6 ml of cold seabass-osmolar PBS 1X.

2.4 Transfer one, two or three eggs to a single concave microscope slide under the microscope with two or three cold PBS 1X drops.

2.5 Dechorionate using watchmaker forceps. Take care not to damage blastula/gastrula.

2.6 Use light mechanical pressure from the forceps to separate blastula from yolk.

2.7 Using a p200 set to 150ul, with a wide-bore tip, transfer blastula to a 1.5ml Eppendorf tube on ice.

2.8 Repeat for required number of eggs for each replicate, pipetting sample up and down.

2.9 Centrifuge on fixed angle centrifuge, 300rfc for 5min @ 4ºC

2.10 Remove supernatant.

The embryos are ready for homogenization and cell isolation for ATACseq protocol from fresh tissue or for formalin fixing for ChIPseq protocol.