AQUA-FAANG - Standard Operating Protocol - Dechorionation of carp embryos, embryo collection and nuclear preparation for ATAC-seq

Overview

This protocol describes a method to remove chorions from carp embryos and to prepare and preserve nuclei from embryos at the desired stages. The resulting nuclei are ready to use for downstream applications such as ATAC-seq.

1. Embryo dechorionation

Materials

- Petri dishes (agarose-coated)
- 500 ml Beakers
- 3ml Paster pipettes

Reagents and solutions:

- 100X E3 medium stock solution: 28,667g NaCl, 1,267g KCl, 4,833g CaCl2 2H2O, 8,167g MgSO4 7H2O in 1l of H2O. Dilute to 1X for working solution and add 0.00001% of methylene blue
- Pronase: 10 mg/ml pronase solution
- Agarose

Preparation of agarose coated petri dishes

1. Prepare and dissolve 1% agarose solution in 1X E3 with 0.00001% methylene blue.
2. Aliquot the agarose solution into petri dishes adding enough volume to cover one-third of the heigh of the petri dishes.
3. Allow the agarose to polymerize. Store agarose-coated plates at 4 degrees and return to room temperature before using with embryos.

Dechorionation procedure

1. After fertilization wait some minutes for chorions to swell up. Carp embryos are sticky so they need to be scraped out of the surface where they are deposited and collected in a petri dish. Alternatively, perform *in-vitro* fertilization directly on a petri dish and wait for the chorions to swell up.
2. Remove the embryo water and replace it entirely with a 10mg/ml pronase solution.

3. Incubate the embryos at room temperature.
4. KEY STEP: During pronase incubation check the embryos under the microscope to observe chorion softening. When some chorions have started to break, the pronase will be ready to be washed.
5. Fill the beakers with E3 medium and immerse the embryos very gently in it without letting the embryos touch the air.
6. Wait for the embryos to fall into the bottom and remove as much medium as possible and repeat the wash in step 5 by gently pouring the liquid through the walls of the beaker.
7. Repeat washing steps (5 and 6) 5 times until the pronase has been completely washed out.
8. Cut the tip of a pasteur pipette until the diameter is big enough to pass one embryo easily.
9. Fill the room-temperature agarose-coated petri dishes with E3 medium
10. Using the pasteur pipetter prepared in step 8, transfer the embryos to the agarose coated petri dishes making sure that just the intact embryos are selected.
11. Wait 10 minutes for the embryos to sit on the plate and remove any possible debreeze or spoilt embryo.
12. Take the dish to an incubator.

Notes:

- The general advice is not to wait until many chorions show cracks or softening as this will lead to overdigestion and embryo death.

- If some chorions remain after this procedure, it is now easy to remove them by gently pulling them apart with dechorionation tweezers. These chorions have to be cleant out of the fish medium as they contain pronase which will affect the embryo development.

- Embryos dechorionated at the 1-cell stage should be re-checked after 5-7 hours when any dead embryos have to be removed to improve the survival of the batch.

- E3 medium has to be changed on a daily basis.

Recommended embryo number per replicate and downstream application

	Late blastula	Gastrula	Early somitogenesis	Mid somitogenesis	Late somitogenesis	Pharyngula
RNA-seq	20	20	10	10	5	5
ATAC-seq	20	10	10	10	5	5
ChIP-seq	40	40	20	20	15	10

2. Nuclear isolation from carp embryos

Solutions to prepare:
Swelling Buffer: 250mM Sucrose, 10mM Tris-HCL (pH 7.9), 10mM MgCl2, 1mM EGTA.
Freezing Buffer: 50mM Tris-HCL (pH 8.3), 40% Glycerol, 5mM MgCl2, 0.1mM EGTA.
Cell Lysis Buffer: **Either**, Nuclei EZ Lysis Buffer [*Sigma N3408*] **or** Swelling buffer + 10% Glycerol and 1% Igepal

1. Collect de-chorionated embryos in a 1.5ml sterile tube. Remove as much E3 medium as possible.

Embryo dissociation and cell swelling:
2. Add 500µl of Swelling Buffer. Dissociate and de-yolk the embryos by vigorous pipetting with a 200µl tip or a pestle and a 200µl tip in case of handling late stage embryos.
3. Pass the embryo solution through a 50µm tube top filter [*CellTrics 04-004-2327*] into a 15ml falcon tube while keeping the tube on ice. Wash the filter with a further 3.5 ml of swelling buffer.
4. Vortex briefly and stand for 5 mins on ice.
5. Spin down at 500g for 5 mins, 4°C and carefully remove (all) supernatant.
6. Resuspend pellet in 200µl Freezing Buffer.
7. Store at -80°C. Samples can be stored for several months in this buffer. Alternatively, freeze in dry ice for 5 minutes and proceed to next step.

Cell lysis and nuclear release:
8. Defrost the solution on ice and add 1 ml Cell Lysis Buffer.
9. Vortex briefly and stand for 5 mins on ice.
10. Spin down at 500g for 5 mins, 4°C and carefully remove (all) supernatant.
11. Resuspend in 1 ml Cell Lysis Buffer and repeat wash (steps 9-11).
12. **QC**: resuspend the pellet in 500µl cell lysis buffer. Transfer 10µl of the solution to a new tube and add 5µl of Trypan blue. Image nuclear recovery efficiency with a cytometer (should see many tiny blue dots [nuclei] and little or none larger cells or cell debris).

Note:
➢ Nuclei can be aliquoted and re-frozen (-80°C) in nuclei EZ lysis buffer (Sigma) for future uses. Aliquots should be thawed on ice and nuclear integrity should be checked before using.
➢ If an excess of cell debris is observed during the trypan blue staining, repeat washes on steps 8-11.