AQUA-FAANG - Standard Operating Protocol - Nuclear isolation from carp embryos

Overview
This protocol describes a method for isolation of intact nuclei from carp embryos. The resulting nuclei are ready to use for downstream applications such as ATAC-seq.

Solutions to prepare:
Swelling Buffer: 250mM Sucrose, 10mM Tris-HCL (pH 7.9), 10mM $MgCl_2$, 1mM EGTA.
Freezing Buffer: 50mM Tris-HCL (pH 8.3), 40% Glycerol, 5mM $MgCl_2$, 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 tube. Remove as much fish water/E3 as possible.

Embryo dissociation and cell swelling:

2. Add 500µl Swelling Buffer. Dissociate and de-yolk embryos by vigorous pipetting with a 200µl tip or a pestle plus 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, 500g, 5 mins, 4°C and carefully remove (all) supernatant.
6. Resuspend pellet in 200µl Freezing Buffer.
7. Store at -80°C, alternatively freeze in dry ice for 5 minutes and proceed to next step.

Cell lysis and nuclear release:

8. Defrost solution on ice and add 1 ml Cell Lysis Buffer.
9. Vortex briefly and stand for 5 mins on ice.
10. Spin down, 500g, 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 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.