SOP nuclear isolation:

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 (works *almost* as well if you can't get hold of Nuclei EZ Lysis Buffer).

1. Collect de-chorionated embryos (ideally 50000 cells are needed for ATAC, it can be dropped to 10000) in 1.5ml tube. Remove as much fish water/E3 as possible.

Brain dissociation and cell swelling:

2. Add 500ul Swelling Buffer. Dissociate and de-yolk embryos by vigorous pipetting with 200ul tip.
3. Pass embryo solution through a 50uM tube top filter [*CellTrics 04-004-2327*] into 15ml falcon tube. Wash filter with a further 3.5 ml of Cell Lysis 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. Re-suspend pellet in 200ul 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 4ml 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. Re-suspend in 4ml Cell Lysis Buffer and repeat wash (steps 9-11).
12. **Optional QC** (re-suspend pellet in 500ul cell lysis buffer. Transfer 10ul of the solution to a new tube and add 5ul of Trypan blue. Image nuclear recovery efficiency with a cytometer (should see many tiny blue dots [nuclei] and no larger cells or cell debris). Remove cells for ATAC (10000-50,000) at this stage.