AQUA-FAANG – Standard Operating Protocol **Nuclei isolation protocol using frozen turbot tissues and cells**

ACKNOWLEDGMENTS

The original protocol for nuclei isolation has been developed by Daniel Macqueen, Rose Ruiz Daniels and Diego Perojil Morata at The Roslin Institute (University of Edinburgh).This protocol has been adapted to turbot biological material by Óscar Aramburu González, Belén Gomez Pardo and Paulino Martínez Portela at University of Santiago de Compostela.

OVERVIEW

This protocol describes a method used to isolate nuclei from frozen turbot tissues (Liver, Brain and Head Kidney) and leukocytes cell cultures, as part of the ATACseq workflow previous to the transposase reaction.

EQUIPMENT

- 6-well dishes
- 70 um cell strainer
- Syringe (no needle)
- Scissors/scalpel (optional)
- 1.5 ml tubes
- 5 ml tubes
- Thermoregulated centrifuge (fixed angle and swinging bucket rotors needed)
- P1000 pipette and tips
- Microscope
- Haemocytometer + cover slips

REAGENTS

- 2X stock of salt-Tris solution (**2X ST buffer**) – 10 ml:
  - 146 mM NaCl (Thermo Fisher Scientific, catalog no. AM9759) (292 ul)
  - 10 mM Tris-HCl pH 7.5 (Thermo Fisher Scientific, catalog no. 15567027) (100 ul)
  - 1 mM $CaCl_2$ Vwr, E506-100 ml) (10 ul)
  - Nuclease-free water (VWR E476-500ml) (9388 ml)
- **1X ST buffer** solution – 5 ml:
  - Dilute 2X ST buffer in nuclease-free water (1:1 ratio)
- Working solution (**TST buffer**) – 4 ml:
  - **2X ST buffer** (2 ml)
  - 1% Tween-20 (Sigma-aldrich, catalog no. P-7949) (120 ul)
  - 2% BSA (New England Biolabs, catalog no. B9000S) (20 ul)
  - Nuclease-free water (VWR E476-500 ml) (1.86 ml)
- Trypan blue

FROZEN TISSUE AS STARTING MATERIAL

1. Place a 6-well culture plate on ice. Don't remove the plate from the ice until the tissue has been homogenized.
2. Place a piece of frozen tissue into a well of the 6-well plate. Add 1 ml **TST buffer** to the well.
3. Use the rubber back of a syringe to mash the tissue. Initially the tissue can be cut up into smaller bits with scissors or a scalpel.
4. Pipette the homogenate and filter through a 40 um cell strainer. Add 1 ml of **TST buffer** to wash the filter.

FROZEN PELLETED CELLS AS STARTING MATERIAL

1. Thaw the frozen cells at room temperature, then place the tubes on ice.
2. Resuspend in 1 ml TST buffer
3. Gently pipette up and down (on ice) for 5 minutes.

COMMON WORKFLOW

1. Bring up the volume to 5 ml with **1X ST buffer**.
2. Transfer the sample to a 5 ml tube and centrifuge at 4ºC for 5 minutes at 500 g in a swinging bucket centrifuge.
3. Remove the supernatant and resuspend the pellet in **1 X ST buffer**, adjusting the volume based on the size of the pellet (100-500 ul). Mix by slow pipetting with a P1000 pipette.
4. Add 10 ul of Trypan blue to an empty 1.5 ml Eppendorf tube. Take a 10 ul sample aliquot for cell counting and mix it with the Trypan blue by pipetting up and down 5 times.
5. Assess the number and integrity of nuclei using an haemocytometer.
6. In a new 1.5 ml tube, dilute the appropriate volume of the original nuclei suspension in 1X ST buffer to obtain 16.5 ul of nuclei suspension (~3000 nuclei/ul, ~50K total nuclei).
7. Continue with the transposase reaction as described in "OmniATAC protocol using Frozen Tissue", from the "Transposase reaction and clean up" section onwards.