PARSE BIOSCIENCES
CUSTOMER DEVELOPED PROTOCOL

Single Nuclei Isolation From Salmon Liver

Developed by Carl I.J. Milton, Erik Burgerhout, and Daniel Macqueen from the Roslin Institute and Nofima; adapted from Rose Ruiz Daniels et. al.

About the Macqueen group and Nofima

The Macqueen group at the Roslin Institute in Edinburgh, UK, has a broad research scope, ranging from evolutionary biology to animal health, with projects in genomics, pathogens, immunology, and other areas. The aquaculture research teams at Nofima investigate how diverse factors, such as temperature or pathogens, affect the phenotype of fish, with a focus on using these findings to improve aquaculture practices.

This protocol was used in

Milton, C. I.J., Taylor, R. S., Sun, J., Lazado, C. C., Wade, N., Burgerhout, E., and Macqueen, D. J. Single nuclei transcriptomics reveals programming of metabolic functions by embryonic temperature in the developing Atlantic salmon liver. Available at https://doi.org/10.1016/j.aquaculture.2026.743685

Additional support resources

This protocol was adapted from:
Ruiz Daniels R, Taylor RS, Dobie R, Salisbury S, Furniss JJ, Clark E, et al. (2023) A versatile nuclei extraction protocol for single nucleus sequencing in non-model species–Optimization in various Atlantic salmon tissues. PLoS ONE 18(9): e0285020. https://doi.org/10.1371/journal.pone.0285020

Materials

Material	Supplier	Part Number	Notes
Centrifuge with swinging bucket rotor	Varies	Varies	Compatible with 15 mL or 5 mL centrifuge tubes and capable of reaching 4°C.
NaCl	Thermo Fisher Scientific®	AM9759	Or equivalent
Tris-HCl pH 7.5	Thermo Fisher Scientific	15567027	Or equivalent
$CaCl_2$	VWR®	E506-100 mL	Or equivalent
$MgCl_2$	Sigma-Aldrich®	M1028	Or equivalent
Nuclease-free water	VWR®	E476-500	Or equivalent
Protector RNase inhibitor	Sigma Aldrich	PN-3335399001	Or equivalent
1% Tween-20	Sigma Aldrich	P-7949	Or equivalent

2% BSA	New England Biolabs®	B9000S	Or equivalent
6 well tissue culture plate	Corning®	CLS3471-24EA	Or equivalent
Cell strainers (40 µm)	Corning	431750	Or equivalent
15 mL conical tubes	Corning	352097	Or equivalent
RNaseZAP® RNase Decontamination Solution	Thermo Fisher Scientific®	AM9780	
Noyes spring scissors	Fine Science Tools	Catalog no. 15514–12	

Preparation

1. Clean the bench top and dissection area with 70% ethanol, followed by RNaseZAP to remove RNases.
2. Prepare the following solutions, and add reagents in the order provided in the table.
3. Clean the Dounce homogenizer with 70% ethanol, followed by deionized water (dH2O). Heat-sterilize the Dounce homogenizer in aluminum foil at 200°C for a minimum of 2 hours. This step can be done in advance, and sterile Dounce sets can be stored in aluminum foil in a clean area.
4. Liver collection:
    a. Dissect the liver tissue from salmon.

b. Flash-freeze tissue in liquid nitrogen.

c. Store at -80°C until ready for nuclei extraction.

2X Salt-tris (ST) Buffer

Reagents	Stock Concentration	Final Concentration	Total Volumes for 10 mL
NaCl	5 M	146 mM	292 µL
Tris-HCl pH 7.5	1 M	10 mM	100 µL
$CaCl_2$	1 M	1.0 mM	10 µL
$MgCl_2$	1 M	21 mM	210 µL
Nuclease-free water			9,388 µL

1X ST Buffer

Reagents	Stock Concentration	Final Concentration	Total Volumes for 6 mL
2X ST buffer			2,997 µL
Nuclease-free water			2,997 µL
Protector RNase inhibitor	40 U/µl	40 U/mL	6 µL

TST Buffer

Reagents	Stock Concentration	Final Concentration	Total Volumes for 4 mL
2X ST buffer			2,000 µL
1% Tween-20	1%	0.03%	120 µL
2% BSA	2%	0.01%	20 µL
Nuclease-free water			1,840 µL
Protector RNase inhibitor	40 U/µl	200 U/mL	20 µL

Procedure

1. Remove samples from the -80°C freezer and keep them on dry ice until ready to begin nucleic acid extraction.
2. Add 1 mL of TST to one well of a 6-well tissue culture plate on ice.
3. Place the frozen sample in the well containing the TST buffer.
4. While in the TST buffer, mince the tissue for 10 minutes using Noyes spring scissors and pipette intermittently with a P1000 set to 500 µL to aid in homogenization.
5. Filter the solution through a 40 µm cell strainer into one of the empty wells in the 6-well plate, and then wash the cell strainer with 1 mL of TST.

Note: Filtration alone may not be sufficient to obtain a suspension that is free from debris. Assess nuclei by visualizing them with a microscope and an appropriate stain. If greater than 5% debris is observed,

perform additional debris removal via filtration, density centrifugation, or magnetic bead separation.

6. Centrifuge nuclei suspension at 500 x g for 5 minutes at 4°C in a 15 mL conical tube.

   Note: Centrifugation conditions may vary between instruments. Users should ensure that all centrifugation steps result in nuclei suitable for analysis before proceeding with fixation and barcoding.

7. Gently aspirate the supernatant until approximately 50 µL of residual volume remains.
8. Resuspend nuclei in 100 µL of 1X ST buffer.
9. Count nuclei and assess quality.

   Note: In our experience, nuclei are much stickier than whole cells. For researchers making the single-nuclei suspension for the first time, we suggest confirming that the sample contains mostly single nuclei. To do this, take 10 µL of the sample and stain it with Hoechst, DAPI, or AOPI for more than 5 min, place it on a hemocytometer, and visualize it under a 20X and 40X objective on an epifluorescence microscope. A best practices resource for hemocytometer use, see: https://www.hemocytometer.org/

10. Proceed to the appropriate Parse Biosciences Nuclei Fixation Kit User Guide (either standard NucleiFixation, Low-Input Nuclei Fixation).

Notice

