

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>

Notice

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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 μ L |
| 1% Tween-20 | 1% | 0.03% | 120 μ L |
| 2% BSA | 2% | 0.01% | 20 μ L |
| Nuclease-free water | | | 1,840 μ L |
| Protector RNase inhibitor | 40 U/ μ l | 200 U/mL | 20 μ L |

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 μ L to aid in homogenization.
5. Filter the solution through a 40 μ m 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 μ L of residual volume remains.
8. Resuspend nuclei in 100 μ L 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 μ L 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).

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