Nuclei isolation from flash-frozen *Pleurobrachia* samples

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– PROTOCOL –

Materials

- ☐ BSA 1 %
- ☐ Buffer, lysis (stock)
- ☐ Buffer, wash (stock)
- ☐ Cell strainer 100 µm
- ☐ Cell strainer Flowmi® 40 µm
- ☐ Dissecting needles
- ☐ Dounce homogenizers & pestels
- ☐ DTT
- ☐ Eppendorfs 0.5 mL
- ☐ Eppendorfs 2 mL
- ☐ Eppendorfs 2 mL low bind
- ☐ Eppendorfs 5 mL
- ☐ Falcon tubes 15 mL
- ☐ Gloves
- ☐ Ice boxes
- ☐ Nuclease-free water
- ☐ PBS for single cell
- ☐ Petri dish
- ☐ Pipettes
- ☐ RNase inhibitor
- ☐ RNaseZap™
- ☐ Samples
- ☐ Scaple blades (new)
- ☐ Tips 1000 µL
- ☐ Tips 1000 µL low retention
- ☐ Tips 200 µL
- ☐ Tips 200 µL low retention
- ☐ Tips 20 µL
- ☐ Tips 10 µL
- ☐ Tweezers

Procedure

> *Make sure to set the temperature of the 4 °C centrifuge beforehand.*
>
> *Always **work on ice**, unless otherwise specified.*
>
> *Always keep the working environment and materials as much **RNase-free** as possible.*
>
> *Place all the working solutions, tubes, and homogenizers on ice before starting.*
>
> *Keep the frozen samples at –80 °C until use. Otherwise, place them in dry ice.*

1. Add **2–5 mL of ice-cold working lysis buffer** into a Dounce homogenizer.
2. Transfer the samples from the tube to the homogenizers using tweezers.

> *If the starting material is too much, place it on a dry-ice-cooled petri dish, and split it in multiple samples using a sterile scaple blade.*

3. Homogenize samples on ice, with a maximum of 10 loose-pestel strokes, and a maximum of 10 tight-pestle strokes.
4. Rinse the pestles back into the homogenizers with **100–500 µL of lysis buffer**.

> *If the sample does not fully dissociat, incubate on ice with the lysis buffer for a couple of minutes.*

5. Filter the homogenate through a 100 µm cell strainer into a 15 mL Falcon tube, using 1000 µL low retention tips.
6. Rinse the cell strainer with **PBS for single cell**.
7. Filter the homogenate through a 40 µm Flowmi® cell strainer into a clean a 15 mL Falcon tube, using 1000 µL low retention tips.
8. Spin samples at 500–900 g for 5 min at 4 °C.
9. Discard the supernatant.
10. Resuspend the nuclei with **2 mL of working nuclei wash buffer**, gently pipetting with 1000 µL low retention tips.
11. Repeat steps 8–10 twice.
12. Resuspend samples in **500 µL of PBS for single cell**.

– RECIPES –

1× PBS with 0.01 % Triton X-100 (PBSTx or PTx)

Compound	Quantity	Molar mass (g/mol)	Final concentration (for 50 mL)
Triton X-100	5.0 µL	647.00	0.01 %
20× PBS	5.0 mL	–	1×
Ultrapure water	–	–	–

1. Add 5 mL of 20× PBS to a graduated cylinder.
2. Add 5 µL of Triton X-100.
3. Fill up to 50 mL with ultrapure water.

6 % goat serum in PBSTx

Compound	Quantity	Molar mass (g/mol)	Final concentration (for 5 mL)
Normal goat serum	300.0 µL	–	6 %
Triton X-100	0.5 µL	647.00	0.01 %
20× PBS	250.0 µL	–	1×
Ultrapure water	–	–	–

1. Add 250 µL of 20× PBS to a beaker.
2. Add 300 µL of normal goat serum.
3. Add 0.5 µL of Triton X-100.
4. Fill up to 5 mL with ultrapure water.

Proteinase K working solution

Compound	Quantity	Molar mass (g/mol)	Final concentration (for 10 mL)
20 mg/mL proteinase K stock solution	1.30 µL	–	2.6 µg/mL
PBST	–	–	–

1. Add 1.3 µL of 20 mg/mL proteinase K stock solution to 8 mL of PBST.
2. Fill up to 10 mL with PBST.

1:500 DAPI staining solution

Compound	Quantity	Molar mass (g/mol)	Final concentration (for 500 µL)
DAPI	1.0 µL	–	1:500
1× PBS	499.0 µL	–	–

– RESOURCES –

1. **IHC in various ctenophore species.**

- Burkhardt, P., Colgren, J., Medhus, A., Digel, L., Naumann, B., Soto-Angel, J. J., ... & Kittelmann, M. (2023). Syncytial nerve net in a ctenophore adds insights on the evolution of nervous systems. *Science*, *380*(6642), 293-297. 10.1126/science.ade5645
- Jager, M., Chiori, R., Alié, A., Dayraud, C., Quéinnec, E., & Manuel, M. (2011). New insights on ctenophore neural anatomy: immunofluorescence study in *Pleurobrachia pileus* (Müller, 1776). *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *316*(3), 171-187. 10.1002/jez.b.21386
- Moroz, L. L., Kocot, K. M., Citarella, M. R., Dosung, S., Norekian, T. P., Povolotskaya, I. S., ...& Kohn, A. B. (2014). The ctenophore genome and the evolutionary origins of neural systems. *Nature*, *510*(7503), 109-114. 10.1038/nature13400
- Sachkova, M. Y., Nordmann, E. L., Soto-Àngel, J. J., Meeda, Y., Górski, B., Naumann, B., ... & Burkhardt, P. (2021). Neuropeptide repertoire and 3D anatomy of the ctenophore nervous system. *Current Biology*, *31*(23), 5274-5285. 10.1016/j.cub.2021.09.005