



**Cryo-preservation of nuclei from tissue for ATAC-Seq using the GentleMACS system (adapted from (Halstead et al., 2020)):**

*(NB: Work quickly using reagents maintained at appropriate temperatures.)*

1. Tissue samples are collected as described: [https://data.faaang.org/api/fire\\_api/samples/ROSLIN\\_SOP\\_Collection\\_of\\_tissue\\_samples\\_for\\_ATAC-Seq\\_and\\_RNA-Seq\\_from\\_large\\_animals\\_20200618.pdf](https://data.faaang.org/api/fire_api/samples/ROSLIN_SOP_Collection_of_tissue_samples_for_ATAC-Seq_and_RNA-Seq_from_large_animals_20200618.pdf).
2. Ideally, keep equal weight between tissue samples (~0.5mm square tissue piece or roughly 200mg)
3. Transfer tissue into a gentleMACS C tube (Mitenyi Biotec Cat# 130-093-237) with 10 ml of Sucrose Buffer.
4. Mince tissue with a scalpel in the gentleMACS C tube (pieces should be cut up to about 1/10 of the size of the square)
5. Homogenize tissue using Mitenyi Biotec gentleMACS Dissociator Program 'm\_muscle\_0.1\_0.1' (equivalent to 'E.01c Tube') twice. Filter homogenate using 100  $\mu$ m Steriflip Vacuum Filter system (Merck Millipore).
6. Bring up to 2.7 mL with Sucrose Buffer.
7. Add 0.3 mL DMSO to samples (10% final concentration), pipetting several times to mix.
8. Aliquot into cryotube vials, freeze at -80°C overnight in Nalgene Cryo 1°C Freezing Container, then move to -80°C freezer or -135°C liquid nitrogen for long-term storage.

**Sucrose Buffer**

<i>Final concentration</i>	<i>Stock concentration</i>	<i>Amount used from stock</i>
250mM D-Sucrose	0.5M D-Sucrose	250 mL
10mM Tris-HCl, pH 7.5	1M Tris-HCl, pH 7.5	5 mL
1mM MgCl <sub>2</sub>	1M MgCl <sub>2</sub>	0.5 mL
Molecular Biology Grade sterile H <sub>2</sub> O to 500 mL		
Filter sterilize with 500 mL 0.2 $\mu$ M Filter System. Store at 4°C. Add Complete Protease Inhibitor		

Tablets (1 per 50mL solution) immediately prior to use.

## References

Halstead, M. M., Kern, C., Saelao, P., Chanthavixay, G., Wang, Y., Delany, M. E., et al. (2020). Systematic alteration of ATAC-seq for profiling open chromatin in cryopreserved nuclei preparations from livestock tissues. *Sci. Rep.* 10, 5230. doi:10.1038/s41598-020-61678-9.