



**Cryo-preservation of nuclei from tissue for ATAC-Seq using the GentleMACS system (adapted from protocol provided by Michelle Halstead 2019 UC Davis):**

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

1. Tissue samples are collected as described: [RI\\_SOP\\_Collection\\_of\\_tissue\\_samples\\_for\\_ATAC-Seq\\_and\\_RNA-Seq\\_from\\_large\\_animals\\_18062020.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 µm Steriflip Vacuum Filter system.
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 µm Filter System. Store at 4°C. Add Complete Protease Inhibitor Tablets (1 per 50mL solution) immediately prior to use.