Single-Cell and Single-Nucleus RNA-Seq Sample Preparation

We recommend preparing between **250,000 – 2,000,000 cryopreserved cells for Single-Cell RNA-Seq**. Higher amounts of cells increase the chance of success. The cell preparation protocols below have been optimized for >2 million cells. If working with <500,000 cells, adjust wash step volumes and tube sizes (i.e. from 15ml conical to 1.5 mL Eppendorf tube) to minimize cell loss.

Cryopreservation

1. Incubate Mr. Frosty or equivalent device at 4°C for a minimum of 1-hour prior to use.

2. For healthy adherent cells lines, enzymatically detach them using trypsin or another enzyme as needed for your specific cell type. For healthy suspension cells, transfer cells in growth media to a conical tube for pelleting.

3. Centrifuge at 500 x g at 4°C to pellet the cells and remove supernatant.

4. Resuspend cells in the appropriate volume of ice-cold cryopreservation solution – 50% FBS/40% growth media/10% DMSO – to achieve a concentration of 4 million cells/mL. If there are less than 2 million cells total, use 500 µL. Transfer 500 µL to a 1.5 mL cryotube on ice.

5. Freeze the cells by transferring the tubes to a pre-chilled Mr. Frosty container or equivalent device, like the one depicted below and place at -80°C.

6. If necessary, an alternate approach is to place the tubes upright in a styrofoam container. Close the styrofoam container with the styrofoam top and then place at -80° C.

For Tissues

If you are submitting tissues for Single-Nucleus RNA-Seq, freeze the tissue according to one of the protocols below. Tissue requirements for are 20 to 50 mg.

Liquid Nitrogen (preferred method)

1. Excise the tissue from the animal and place in a microfuge tube.
2. Submerge in liquid nitrogen for 2 minutes.
3. Store at -80 °C.

Dry Ice

1. Excise the tissue from the animal and place in a microfuge tube.
2. Place tube into a dry ice bath with ethanol for 15 minutes.
3. Store at -80 °C.

For Organoids and Very Small Tissues (<10mm^3)

Organoids and very small tissues (eg. pancreatic islets, embryonic tissues, tissues only several cell layers thick such as retina or epidermis) should be cryopreserved in 500 µL of cryopreservation solution – 50% FBS/40% growth media/10% DMSO as if they were cells, aiming for 1-2 million cells total.