CitraPrep protocol

Prepare stock buffers (good for 1month at 4 degrees):

CitraHB:
- 0.25M sucrose
- 25mM KCl
- 5mM MgCl2
- 20mM Tricine-KOH pH 7.8
- 10mM citric acid

Diluent:
- 150mM KCl
- 30mM MgCl2
- 120mM Tricine-KOH pH 7.8
- 10mM citric acid

Detergent:
- 50uL Igepal CA-630 in 950uL CitraHB

Make working solutions on ice:

Make fresh each time:
- 1M DTT (1000X)
- 0.15M spermine (1000X)
- 0.5M spermidine (1000X)

50% iodixanol:
- 5 volumes OptiPrep
- 1 volume Diluent

40% iodixanol:
- 3.2 mL 50% iodixanol

30% iodixanol:
- 4.5mL 50% iodixanol

Make an aliquot of 10mL CitraHB buffer *complete*, add 1 mini protease inhibitor tablet and dissolve

Add spermine, spermidine, and DTT to all buffers for 1X concentration

Add 0.5 U/uL SUPERase RNase inhibitor to all buffers
Add 0.5 U/uL RNAsin to all buffers
(total 1U/uL RNase inhibitor)

Per sample, you will need 1mL SUPERasin and 500uL Protector.

Make nuclei resuspension buffer:
- 1% BSA
- 1x PBS
- 0.5 U/uL both RNase inhibitors
  Do not add spermine, spermidine, or DTT to this buffer, will interfere with cDNA synthesis

Protocol:

1. Put 7mL glass dounce homogenizer on ice to cool

2. Use clean scissors to cut snap frozen tissue into small pieces (less than 0.5cm) in a petri dish on ice in 1mL CitraHB buffer *complete*

3. Transfer to glass dounce homogenizer on ice, add 4mL CitraHB buffer *complete* and incubate for 7 minutes on ice

4. Homogenize 20x with loose pestle, add 320uL of Detergent and homogenize 20x with tight pestle. Make an aliquot to analyze nuclei and add more homogenization if needed

5. Filter through 40uM strainer

6. Add 5mL 50% iodixanol solution to the 5mL CitraHB and vortex to mix

7. Underlay sample with 30% and 40% iodixanol solutions

8. Weigh sample and centrifuge for 18 min at 10,000g at 4 degrees with no brake

9. Collect nuclei from interface layer with a 1000mL pipette, place in new tube.

10. Add 10mL of nuclei wash buffer in a 15mL tube, vortex

11. Filter through 20uM filter

12. Spin for 10 min at 500g at 4 degrees to pellet nuclei

13. Resuspend nuclei in 10mL of nuclei wash buffer, repeat spin

14. Resuspend nuclei in 200uL of nuclei wash buffer, count nuclei using hemocytometer

15. Load for single cell