Nuclei isolation for 10X single nuclei barcoding

We obtain fresh frozen brain blocks from the Edinbrugh Brain bank and cryosection them at 20um. We use about 20 of those sections for nuclei isolation. Usually we are only interested in white matter which we scrape of the slides using flexible razor blades. We get the tissue we need out of the freezer, air dry it in a hood and while they dry, we prepare the buffers.

Nuclear Isolation with NUC201 isolation kit from Sigma

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Preparation:

1. Prepare PURE Lysis Buffer (200ul per sample): for 8 samples: 1700 ul PURE buffer, 17ul 0.1M DTT, 17 ul 10% Triton, 34ul RNAseinhibitor
2. Prepare sucrose cushion: (560 ul per sample): for 8 samples: 5ml PURE 2M Sucrose solution, 550ul Sucrose Cushion buffer, 55ul 0.1M DTT, 100ul RNAseInhibitor

Procedure:

1. Homogenize the tissue in 200ul PURE lysis buffer using syringes with descending needle size (last used are insulin needles).
2. Mix each 200ul lysate with 360ul sucrose cushion and keep on ice
3. Filter through a 30um filter
4. Add 200ul of sucrose cushion to an Eppendorf tube and carefully overlay with 560ul mixture of step 3
5. Centrifuge for 45min at full speed (cooled down table top centrifuge)
6. Remove the supernatant (pellet should be visible)
7. Re-suspend in PURE storage buffer (200ul per sample) + 0.2U/ul RNASE-Inhibitor
8. Centrifuge for for 5min at 500g
9. Re-suspend in PURE storage buffer (200ul per sample) + 0.2U/ul RNASE-Inhibitor
10. Centrifuge for for 5min at 500g
11. Remove supernatant but CAVE! Keep supernatant and measure free DNA using a NanoDrop.
12. Re-suspend pellet in 50ul Storage buffer + 0.2U/ul RNASE-Inhibitor
13. Count nuclei using the BioRad cell counter (in flow cytometry lab. You will need Trypan blue and counting slides. If you want to save images bring a USB stick along).
14. Jump up and down with joy that you have made it through!