Rapid Nuclei Isolation from Human Brain

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Abstract

This protocol outlines our preparation of single-cell suspension from surgically acquired fresh human adult brain tissue. This method is adapted from Habib et al[1].

1 Habib, N. *et al.* Massively parallel single-nucleus RNA-seq with DroNc-seq. *Nat Methods* **14**, 955-958, doi:10.1038/nmeth.4407 (2017).

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Protocol

Step 1.

Prepare Nuclei Suspension Buffer (NSB):

For drop-seq: 1x PBS, 0.01% BSA (ultra pure), 0.2U/ul RNAse inhibitor

For 10X Genomics: 1X PBS, 1%BSA (ultra pure), 0.2U/ul RNAse inhibitor

Step 2.

Dounce homogenize tissue samples (smaller than 0.5 cm) or cell pellets in 2 ml of ice-cold Nuclei EZ lysis buffer (Sigma, #EZ PREP NUC-101). Dounce tissue 20-25 times with pestle A, followed by 20-25 times with pestle B while on ice.

Step 3.

Move sample to a 15 ml conical tube, add 2 ml of ice-cold Nuclei EZ lysis buffer and incubate on ice for 5 min.

Step 4.

Collect nuclei by centrifugation at 500 x g for 5 minutes at 4°C. Discard supernatant and carefully resuspend nuclei in 4 ml of ice-cold Nuclei EZ lysis buffer. Incubate on ice for 5 minutes.

Step 5.

Repeat the centrifugation at 500 x g for 5 minutes at 4°C. Resuspend isolated nuclei in 1 ml of NSB, and filter through a Flowmi Cell Strainer (Bel-Art, H13680-0040).

Step 6.

Stain 10µl of nuclei suspension with DAPI (1:5000) or 0.4% Trypan Blue to count.

Step 7.

Adjust the volume with NSB to 1000 nuclei/µl (for 10X Genomics) and 300 nuclei/µl (for drop-seq).