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Single-nucleus RNA (snRNA) sequencing

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Protocol status: Working
We use this protocol and it's working

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Abstract

Step-by-step protocol describing the snRNA sequencing workflow for nuclei isolated from post-mortem human brain tissue.

Nuclei isolation from human brain tissue

5h

1 Isolate nuclei from human brain tissue following the protocol we have previously published:

5h

Protocol

NAME

Nuclear Extraction from Tissue for FACS

CREATED BY

Raquel Garza

Preview

Single nuclei preparation for sequencing

30m

2 Load the single-nuclei suspension (8500-10000 nuclei/sample) onto the Chromium Next GEM Chip G Single Cell Kit together with the reverse transcription mastermix following the manufacturer's instructions. The protocol for the Chromium GEM single cell 3' kit to generate single-cell gel beads in emulsion can be found on the 10X Genomics website (10X Genomics, PN-1000268). It is possible that new kits and chemistries are released by 10X Genomics. In that case, it can be relevant to use the latest version of the kit and of the protocols provided by the manufacturer.

30m

3 Amplify cDNA following the guidelines from 10X Genomics. We used 13 cycles of amplification of the 3' libraries.

4 Generate sequencing libraries with unique dual indices (TT set A).

snRNA sequencing

3d

5 Pool the libraries from the previous step for sequencing on an Illumina sequencer (we sequenced either on a Novaseq6000 or on a Novaseq X Plus) using a 100-cycle kit and 28-10-10-90 reads.

3d