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Nuclei Extraction from Flash-Frozen Grafted Mouse Brains for 10X Genomics Single-Cell Sequencing

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SOX6 mDA differentiation

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

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Abstract

This protocol explains how to extract and process nuclei from the dorsal striatum of flash-frozen mouse brains containing grafts for single-nucleus RNA-sequencing using 10X Genomics technology.

Materials

- Dry-ice chilled isopentane
- -80°C freezer
- -20°C freezer
- -20°C cooled chamber (Cryostat for example)
- Brain matrix
- Chilled razor blades
- 1 mm biopsy punchers (PFM Medical, 49101)
- 10X Genomics recommended Tips (LTS 200UL Filter RT-L200FLR)
- 10X Genomics Chromium Nuclei Isolation Kit (Cat. No. 1000494)
- 10X Genomics Chromium GEM-X Single Cell 3' Kit v4 (1000691)
- Chromium GEM-X Single Cell 3' Chip Kit v4 (1000690)
- Dual Index Kit TT Set A (1000215)
- 10X Genomics recommedend LoBind Eppendorf tubes (2 mL, 0.5 mL)
- 1% Nuclease-free BSA solution
- Sterile filtered PBS 1X

Troubleshooting

Safety warnings

Remember to check your local policies when working with human samples.
In our case, our fresh human cells had to be worked in BSL2-compliant rooms.

Ethics statement

This protocol requires prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

In our case, all procedures were approved by the local ethical committee (Stockholms djurforsoksetiska namnd) and followed the EU directive 2010/63/EU under the ethical permit of Ernesto Arenas Cases and Sandra Gellhaar (12265-2021; 2-3143/23; 2-3595/24; 2-3147/24).

Before start

All working surfaces and equipment have to be treated and handled for RNA extraction, for example, cleaned with RNAse-away for RNAse decontamination and RNAse-free disposable elements like the eppendorf tubes.

Brain Collection and Storage

15s

1 **Euthanasia**: Euthanize animals via cervical dislocation.

2 **Brain Extraction**: Promptly extract the whole brain immediately after confirmed death, using scissors and forceps to break open the skull, and spatula to remove the brain.

2.1 Decapitate mouse using sharp scissors

2.2 Use scissors or scalpels to open the skin and expose skull

2.3 Use the scissors or bone rongeurs to cut the skull between the eyes, and into the ears from the back

2.4 Use the blunt forceps to lift the detached skull, exposing the brain

2.5 Remove the brain using a spoon or a spatula by slowly detaching the nerves from the brain.

3 **Flash-Freezing**: Submerge brains in dry-ice chilled isopentane for **10–15 seconds** to flash-freeze.

15s

4 **Storage**: Transfer flash-frozen brains to **-80°C** for storage until further processing. Liquid nitrogen works for very long term storage.

Tissue Sectioning and Punch Collection (Day of Nuclei Extraction)

5 **Equilibration**: Equilibrate brains to **-20°C** before slicing.

6 **Sectioning Setup**: Place brains in a -**20°C cooled slicing chamber**. Stabilize using a brain matrix.

7 **Slicing**: Slice brains into four 1-mm thick coronal sections. **2 slices anterior and 2 slices posterior to the center of the graft implantation site**. Use chilled razor blades for clean

cuts.

8 **Tissue Punching**: Using 1 mm biopsy punchers, punch dorsal striatum punches (where graft is expected) from each slice. Collect punches into pre-prepared tubes (see below, step 9).

Nuclei Dissociation

9 Tube Preparation: Pre-coating

9.1 Prepare 1% Nuclease-free BSA in sterile nuclease-free PBS1X

9.2 Pre-coat 2 and 0.5 mL LoBind Eppendorf tubes with **1% BSA overnight at 4°C**.

9.3 Before use, briefly rinse tubes with sterile-filtered 1X PBS.

10 Perform nuclei isolation using the 10X Genomics Chromium Nuclei Isolation Kit (1000494). Follow the manufacturer's instructions for optimal results.

11 It is recommended to continue the GEM preparation inmediately