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Olfactory epithelium tissue harvest, dissociation, and nuclei extraction

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We use this protocol and it's working.

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Abstract

Protocol for harvesting olfaactory epithelium from mice rapidly, and subsequently processing the tissue for single-nucleus sequencing applications.

Olfactory epithelium tissue Harvest

1 Begin by deeply anesthetizing the mouse and transcardially perfusing with at least 10 mL of ice-cold 1X PBS.

2 Remove the skin to expose the skull.

3 Using a pair of fine scissors, make two incisions to break the arches from the squamosal bone bilaterally.

4 Make a shallow incision from orbit to orbit, along the most anterior aspect of the frontal bone (incise only the dorsal surface of the frontal bone, being careful not to cut any underlying structures or bones that comprise the orbit).

5 Cut along the midline of the skull from the foramen magnum, through the sagittal and interfrontal sutures, until the interorbital incision made in the frontal bone is reached.

6 Insert a pair of forceps into the incision made along the midline of the skull and separate one hemisphere of the cranial vault. This action will also remove the bones of the orbit (frontal, maxillary, and lacrimal), exposing the lateral aspects of the OE. Take care to not inadvertently damage the OE from this point forward.

7 Holding the nasal bone and incisor teeth, break away the contralateral cranial vault. Allow the brain (including the olfactory bulbs) to be removed with the hemisphere of bone as it is torn away. The only visible bony structures that should remain after this step are the base of the skull and the bones surrounding the OE.

8 Using a pair of bone nippers or rongeurs, remove any remaining frontal bone and premaxillary bone up to the base of the incisor teeth.

9 Use bone nippers to cut along the frontomaxillary suture, taking care to not damage the underlying exposed OE. Lift the nasal bone off the cribriform plate, exposing the OE.

10 Using fine-tipped forceps, gently secure the dorsal aspect of the nasal septum at its junction with the cribriform plate and pull posteriorly. With minimal resistance, the main olfactory epithelium should break free as an intact structure.

Olfactory epithelium dissociation and nuclei isolation

11 Place one C-tube (Miltenyi Biotec, cat #130-093-237) and two 15 mL conical tubes (Celltreat cat #229491), per sample, on ice.

12 Prepare lysis buffer by adding RNAse inhibitor (to a final concentration of 0.2 U/µL) to the pre-made nuclei extraction buffer (Miltenyi Biotec cat #130-128-024).

13 Add 4 mL of 1% BSA to each 15 mL conical tube, vortex briefly, and discard excess. This minimizes nuclei adhesion to the tube.

14 For whole OE, add 2 mL ice-cold lysis buffer to each C-tube.

15 Add OE samples to the C-tubes. Using #2 forceps, hold each sample over its respective C-tube and cut it into small pieces using a pair of fine tip scissors.

16 Place the C-tubes containing each sample upside-down onto the GentleMACS tissue dissociator (Miltenyi Biotec, cat #130-093-235). On the GentleMACS dissociator screen, the square corresponding to each block will change from **Free** to **Selected**.

17 Press the folder icon to cycle into the **Miltenyi** folder. Using the arrows, scroll to the **4C_nuclei_1** program (a preset program within the dissociator). Press **OK** to apply the program to each block, and then press **Start** to begin the cycle. Remove the C-tubes from the dissociator immediately after cycle completion. NOTE: it is important to remove the samples from the dissociator **immediately** after cycle completion. and proceed with the next steps of the protocol to avoid over-lysis of the nuclei.

18 With a pipette, aspirate the homogenized suspension from the C-tube and filter it through a 70 µm strainer (Miltenyi Biotec, cat #130-110-916) into the first BSA-coated conical tube. Avoid aspirating the bone fragments that have accumulated at the bottom of the C-tube.

19 Rinse the strainer with 1 mL of ice-cold 1X PBS.

20 Centrifuge the filtered suspension at 500 × *g* for 5 minutes at 4 °C in a swinging-bucket centrifuge.

21 Gently aspirate and discard the supernatant; avoid disturbing the pellet. Resuspend the pellet in 1 mL of ice-cold 1X PBS by gentle pipetting.

22 Pass the resuspended nuclei through a 30 µm strainer (Miltenyi Biotec, cat #130-110-915) into the second BSA-coated conical tube. The concentration of nuclei can be quantified using a hemocytometer or an automated cell counter.

23 Proceed immediately with the chosen downstream protocol (with or without FACS) to preserve sample integrity and prevent degradation.

Guidelines and Warnings

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