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Nuclei Isolation for SnRNA-seq and SnATAC-seq from Frozen Fresh Human Retina Sample

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

The protocol of single nuclei RNA/ ATAC sequencing describes the isolation of nuclei from the human flash frozen retinal tissue. It highlights two major sections:

- the dissection technique for the fresh retina tissue,
- a nuclei isolation protocol which results in a single cell suspension with high quality,

Depending on tissue quality, optimization might be needed for some steps, including the homogenization, reagent/time of lysis, centrifugation speed/time, and filtration steps.

MATERIALS TEXT

Petri dishes 10cm
2mm disposable punches (Milex Disposable Biopsy Punch, REF 33-31)
4mm disposable punches (Milex Disposable Biopsy Punch, REF 33-34)
Dissection scissors (Micro Dissecting Scissor 4 1/2", BRI 25-1000)
VANNAS scissors (VANNAS Extra-Special Micro Fine scissor, 3" straight, BRI 11-1000)
Dissection forceps serrated(Micro Dissecting Forceps 4" serrated, BRI 10-2320)
Dissection forceps (Pattern #55, BRI 10-1050)
Blades (Scalpel Baldes #11, BRI 26-1315)
Razor blade (VWR, Cat. NO. 55411-050)
Kimwipes or gauze
Dewar of liquid nitrogen
2ml Punch tubes
15ml Falcon tubes

Lysis buffer:
10 mM Tris-HCl 50 ul (Sigma T2663-1L)
10 mM NaCl 10 ul (Sigma S6546-1L)
3 mM $MgCl_2$ 15 ul (Ambion The RNA Company 102R33A)
0.1% NP-40 12.5 ul
Nuclease-free H_2O 4912.5 ul (Ambion The RNA Company AM9937)

1 ml Dounce Tissue Grinder (WHEATON 357538)
40um Flumi Cell Strainers (H13680-0040)
Nuclei Buffer* (20X) (10x Genomics, PN 2000153/2000207)

BEFORE STARTING

For a better quality of nuclei, frash frozen the tissue in liquid nitrogen and never thaw the tissue before douncing in cold Lysis Buffer.
It is important to mince the tissue as small as possible on dry ice before adding Lysis Buffer.
Prepare Lysis Buffer fresh, maintain on wet ice.
Incubation time need to be optimized according to different tissue types. Prolonged lysis time could result in over-lysis.

Human Fresh Eye Collection and Dissection

1 Remove optic nerve stalk with blade and orbital fat with forceps from the eye. Place the eye in a vial filled with 1X PBS. Label the vial with all essential information. Note: For best quality, only eyes within 6 hr post-mortem should be used.

2 The eyes are kept on Kimwipes wet by PBS in a dish and measure its sagittal, transverse, and axial diameters using a caliper.
Place the eye in a cuvette to take fundus and OCT images.

3 Place the eye on a folded wet Kimwipe or a piece of wet gauze to keep it stable. Gently make an incision just beneath the limbus with a new blade under the microscope, use a serrated forceps to keep the eye stable.

4 Use dissection scissors to cut all the way around the limbus and remove the anterior segment (cornea, lens, and iris). Separate the lens, cornea, and iris with forceps, and place them into different tubes. Take note if the lens is artificial.

5 Make 4 cuts with dissection scissors between the attachment points of the eye muscles, and flatten the eye such that it consists of 4 separate lobes of tissue. Carefully trim away as much vitreous as possible without disturbing the retina using VANNAS scissors .

6 With a 2mm punch biopsy tool centered over the fovea, press down to make an incision. With a 4mm punch biopsy tool centered over the macula, press down to make an incision. Carefully transfer the fovea and macula (no RPE or other tissues) into separate 2ml Punch tubes. Label and place the tubes in liquid nitrogen immediately and store at -80°C overnight.

7 Use a razor blade to make an “X” to separate the lobes, and number the lobes clockwise as 1-4, starting with the lobe containing the macula. For each lobe, separate the retinal and RPE/choroid layers using forceps carefully. Store each sample (4 retinal lobes and 4 RPE/choroid lobes, totally 8 samples) in a 2ml tube. Label and place the tubes in liquid nitrogen immediately and store at -80°C overnight.

8 Collect the remaining tissue in a 15ml Falcon tube. Label and place the tube in liquid nitrogen immediately and store at -80°C overnight. This tissue can be used for DNA extraction.

Nuclei Isolation

9 Disinfect the bench with 70% ethanol and clean up the work area. Rinse tools in water before placing them in a container with 70% ethanol.

10 Cool on ice the following items: razor blades, glass dounce homogenizer (1 ml), and RNAse-free lysis buffer.

Using the pre-cooled razor blade, cut a block (size of rice grain) of frozen retina in a dish on dry ice, and mince the tissue as small as possible.

11 Add 500 ul ice-cold RNase-free lysis buffer on wet ice. Start the timer.

12 Pipet the tissue in lysis buffer 10 times with a P1000 pipet (set to 500ul) to disrupt the tissue on wet ice. Transfer the tissue to the precooled glass dounce.

13 Dounce the sample 10 times with loose pestle, and 10 times with tight pestle, slowly and gently while avoiding the generation of bubbles.

14 Leave on ice for 3 min total.

15 Filter the sample through 40um Flumi Cell Strainers into a 2ml ice-cold Eppendorf tube.

16 For snRNA-seq, add DAPI (1ug/ml) and then FACS sort for nuclei immediately. Count the nuclei concentration. The final concentration should be 800-1000 nuclei/ul. The nuclei are ready for 10X snRNA-seq.

For snATAC-seq, spin down the sample at 500 g for 5 min at 4°C. Remove the supernatant without disturbing the pellet and resuspend the pellet in 50 ul diluted Nuclei Buffer (1X). Stain the nuclei with trypan blue and count the concentration. The final concentration should be 3000-5000 nuclei/ul.

17 Proceed to 10X snRNA-seq/10X snATAC-seq.