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Protocol for Preparing Brain Samples for MUSIC V.1

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Wenxin Zhao[1], Sheng Zhong[1]

[1]Shu Chien-Gene Lay Department of Bioengineering, University of California San Diego

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Wenxin Zhao
Shu Chien-Gene Lay Department of Bioengineering, University ...

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We use this protocol and it's working

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Abstract

Here states the detailed procedure to prepare brain samples for MUSIC study.

Troubleshooting

Tissue pulverization and crosslinking

1 Cut a portion of post-mortem human brain frontal cortex sample on dry ice with heavy razor blades, and collect 50 mg of the sample in a 1.5 mL LoBind tube.

2 Thaw the 50 mg of brain sample on ice, and chop the tissue into smaller pieces by pestle. Store the rest of the sample at -80°C.

3 Incubate the sample with 10 mL of 2 mM disuccinimidyl glutarate (DSG) in 1X PBS in a 15 mL LoBind tube at room temperature for 45 min with gentle rotation.

4 Wash once with 10 mL of 1X PBS by centrifugation at 1,000 x g for 4 min.

5 Resuspend the sample in 15 mL of 1X PBS containing 3% formaldehyde, and incubate for 10 min with a gentle rotation.

6 Quench the crosslinking reaction by the addition of 5 mL of 1.25 M glycine followed by an incubation of 5 min with a rotation.

7 Centrifuge the sample at 1,000 x g for 4 min, and wash the sample twice with ice-cold 1X PBS containing 0.3% BSA (wt/vol).

Nuclei isolation

8 Use Chromium Nuclei Isolation kit (10X genomics, 1000494) to isolate nuclei from crosslinked cortex samples.

9 Transfer 50 mg frozen tissue into pre-chilled sample dissociation tube.

10 Add 400 µL of Lysis Buffer to Sample Dissociation Tube. Dissociate tissue with plastic pestle until homogeneous.

	A	B
	Component	**Volume (µL)**

	A	B
	Lysis Reagent	1000
	Reducing Agent B	1
	Sufactant A	10
	Total Volume	1011

Lysis Buffer

11 Add 600 µL of lysis buffer into the tube, and mix 10 times by pipetting. Incubate on ice for 10 min.

12 Equally load the solution into two nuclei isolation column, and centrifuge the tubes at 16,000 x g for 20 sec at 4°C.

13 Vortex the flowthrough in the collection tube that contains nuclei for 10 sec at 3,200 rpm or max speed to resuspend nuclei.

14 Centrifuge the collection tubes for 3 min at 500 x g at 4°C to pellet nuclei. Carefully discard the supernatant.

15 Resuspend the nuclei in 500 µL of Debris Removal Buffer provided by the kit by pipetting 15 times.

	A	B
	Component	**Volume (µL)**
	Debris Removal Reagent	500
	Reducing Agent B	0.5
	Total Volume	500.5

Debris Removal Buffer

16 Centrifuge the nuclei at 700 x g for 10 min at 4°C. Carefully discard the supernatant.

17 Resuspend the nuclei in 1 mL of Wash and Resuspension Buffer.

	A	B
	Component	**Volume (µL)**
	1X PBS	1750
	10% BSA	200
	RNase Inhibitor (40X)	50
	Total Volume	2000

Wash and Resuspension Buffer

18 Centrifuge the nuclei at 500 x g for 5 min at 4°C. Carefully discard the supernatant.

19 Resuspend the nuclei again in 1 mL of Wash and Resuspension Buffer.

20 Centrifuge the nuclei at 500 x g for 5 min at 4°C. Carefully discard the supernatant as much as possible.

21 The nuclei are subjected to nuclei counting and the following procedures.