Nuclei isolation from human skeletal muscle

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Working

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ABSTRACT

This protocol describes a methond of single nucleus isolaton from human skeletal muscle for snRNA sequencing.

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

1 Prepare 5ml Buffer A and 10ml Buffer B for each human sample

2 Dissect all the hind limb muscles and place in a 35/60mm tissue culture dish with 0.5ml PBS.

3 Remove the fat from muscle using a scissors, and wash the muscle with 1X PBS twice.

4 Mechanically chop the muscle into 1mm pieces with a razor, mix the red and white muscles.

5 Rinse the 7 ml dounce homogenizer with MiliQ water.

6 Transfer the 0.3g of the mashed muscle into a 7 ml dounce homogenizer with 3ml of Buffer A using the razor (the rest of the muscle will be used for single cell isolation).

7 Ground the tissue on ice for 50 strokes with the loose Pestle A (clearance of 0.114 mm), try to avoid bubbles. Do not twist the pestle

while pushing the pestle down. It might cause the nuclei degradation.

8 Filter the mixture passed through a 100micron strainer on top of a 50ml falcon tube. Rinse the dounce homogenizer with 1ml Buffer A and then wash through the cell strainer, collect into the 50ml falcon tube.

9 Repeat the washing with another 750ul of Buffer A. The combined tissue homogenate in the 50ml falcon tube now should be ±4.75ml.

10 add 250ul of Triton X-100 (10%) to the tissue homogenate to make the final concentration of Triton X-100 is 0.5%. Mix the solution gently by pipetting.

11 Wash the 7 ml dounce homogenizer with MiliQ water for the next step.

12 Placed the tissue homogenate back into a cleaned dounce homogenizer.

13 Ground the mixture an additional 50 strokes with the tight Pestle B (clearance of 0.076mm).

14 Filter the mixture through a 40micron strainer. Leave the bubbles in the strainer and collect the filtered solution that accumulated on the bottom of the strainer.

15 Centrifuge the tissue filtrate at 3000 x g for 5min at 4 degrees to get nuclei pellet. Remove the supernatant entirely.

16 Re-suspend the nuclei pellet in 1ml Buffer B and transfer to a 1.5ml Eppendorf tube.

17 Prepare 4 new Eppendorf tubes, add 475 ul of Buffer B into each tube.

18 Gently mix the nuclei suspension by pipetting, and then take 225 ul of nuclei suspension and 300 ul of Percoll solution into each tube to create a 27% Percoll-nuclei solution. Mix by pipetting.

19 Centrifuge the Eppendorf tube at 20,000 x g for 15 minutes at 4 degrees, remove the supernatant and keep only the nuclei layer (pellet), note the tube orientation.

20 Re-suspend the nuclear layer into 200ul of Buffer B. Pool the nuclei suspensions from 4 tubes to one tube.

21 Spin down the nuclei at 2500 x g for 3 minutes at 4C.

22 Remove the supernatant and add 1ml of buffer B. Gently disperse the pellet with pipetting and spin down the nuclei at 2500 x g for 3 minutes at 4C.

23 Remove the supernatant and re-suspend the nuclei in 50-200 ul of Buffer B (the volume need to be adjusted according to the size of the pellet).

24 Stain the intact nuclei using trypan blue (50:50) and quantify by counting with a hemocytometer.

25 Wash the pestles and homogenizers with water-80% EtOH and leave to dry out till next experiment.