Mar 16, 2025

Nuclear prep and FACS for 10x Genomics Chromium single-nucleus sequencing

DOI
dx.doi.org/10.17504/protocols.io.261genm5yg47/v1

Matt Keefe[1]
[1]UCSF

NOW Lab Protocols

Matt Keefe

DOI: dx.doi.org/10.17504/protocols.io.261genm5yg47/v1

Protocol Citation: Matt Keefe 2025. Nuclear prep and FACS for 10x Genomics Chromium single-nucleus sequencing. **protocols.io https://dx.doi.org/10.17504/protocols.io.261genm5yg47/v1**

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

Created: July 18, 2022

Last Modified: March 16, 2025

Protocol Integer ID: 67006

Funders Acknowledgements:
NIH
Grant ID: 1RF1MH121268-01

Abstract

Protocol is used for nuclear isolation and single cell RNA sequencing in the Nowakowski lab.

Introduction

1 **Nuclear prep and FACS for 10x Genomics Chromium single-nucleus sequencing**
(protocol version 1.3.2, J. Brown, Arlotta lab)

2 Partially from Habib et al., *Massively parallel single-nucleus rna-seq with dronc-seq*, Nature Methods 2017. (With additional commentary by Eugene Drokhlyansky and Ehsan Habibi, Regev lab. Per Gene, this method for nuclear extraction works best. Per Ehsan, this method works very well for brain)

Reagents

3
- EZ Lysis Buffer from Nuclei EZ Prep kit (Sigma, Cat # NUC-101)
  ensure that the lysis buffer is the version designed for cell culture, and not the version designed for tissue
- 2ml glass dounce tissue grinder (e.g., Sigma, Cat # D8938)
  if using a different product, ensure pestles have ball end and not U-end, or they will damage the nuclei
- 35-µm cell strainer tube (e.g., Corning, Cat # 352235)
- 1ml pipettor and pipette tips (note: 2ml serological pipettes do not fit in the dounce)

Nuclei Suspension Buffer (NSB):
- 1 × RNAse-free molecular-biology grade PBS
- 0.1% molecular-biology grade BSA (100 µg/ml) (e.g., NEB B9000S)
- 0.2U/µl RNase inhibitor (e.g., Clontech, Cat # 2313A)

For 5 ml:
- 5 ml PBS
- 25 µl NEB BSA (@ 20 mg/ml)
- 25 µl RNase inhibitor (@ 40U/ul)

Tissue harvesting and storage

4 Dissect tissue and place in 1.5 mL microcentrifuge tube, transferring as little media as possible. If necessary, cut tissue samples into pieces <0.5 cm first. Flash-freeze on dry ice and store at -80^{o}C.

5 Dissect the region of interest, place on the inside wall of a 1.5 mL tube, make sure it doesn't slide down, and freeze tube in dry ice. The sliding part is to make sure there is no excess liquid on the tissue. You don't want to pat dry, but you don't want tissue to freeze in any liquid.

Nuclear prep

6 **Note: Maintain RNAse-free conditions throughout. Keep nuclei ice-cold at all times. Resuspend and mix gently to avoid excessive damage.**

7 Preparation

7.1 Wash dounce and pestles with 100% EtOH, followed by RNAse Zap. Wash in 2-3 rounds of RNAse-free water (in 50 ml conical), and rinse with EZ Lysis Buffer.

7.2 Chill dounce and pestles on ice (pestles in a 50 ml conical tube).

7.3 Prepare ~5 ml Nuclei Suspension Buffer per sample (plus ~1-2 ml extra if doing FACS) and place on ice.

7.4 If you are using the Vybrant DyeCycle Ruby stain, make ~1 ml NSB & Ruby (1:500) per sample (or a bit extra if you think you might need to dilute the sample). Add an additional 5 µl / ml RNAse inhibitor to this solution (final 0.4U/µl), because the nuclei will be sitting in it for a while. Hold on ice.

The Ruby is provided in a DMSO solution and is not very soluble in cold media; if necessary, dilute it 1/10 in a small amount of room-temperature NSB, then 1/50 into the cold NSB (1:500 final).

7.5 Prepare ~100 µl of rich-NSB to sort the cells into: 100 µl NSB (without Ruby) with 5 µl extra RNAse inhibitor (final 2U/µl) and 5µl extra BSA (final 1%). Hold on ice.

7.6 Plan to sort into a 96-well plate; pick one well per sample and coat the entire inside with ~20 µl of the NEB BSA (undiluted), then fill with NSB. Place the plate in a clean ziploc bag to transport it to/from the core, and hold on ice. (The BSA coating step reduces adherence and improves recovery.)

7.7 Before sorting, draw off the BSA in the wells and rinse the well 1-2 times with ~200 µl ice-cold NSB (the basic one), then fill with ~10-20 µl of rich-NSB (based on the volume you expect to collect – try to keep the final post-sort volume under 30 µl for the v2 kit, or 45 µl for the v3 kit). Try to make sure there are no bubbles.

7.8 Pre-chill centrifuge.

7.9 lace a 15-ml conical tube on ice for each sample.

8 Transfer tissue to chilled dounce. Do not allow tissue to thaw. For pieces of mouse cortex under 0.5 cm, it is not necessary to chop the tissue. Immediately add 2 ml ice-cold EZ Lysis Buffer.

9 Homogenize with 20 strokes of pestle A and 20 strokes with pestle B. Raise and lower slowly, avoiding bubbles. Keep nuclei on ice at all times.

10 Transfer suspension to chilled conical (from step 2). Rinse dounce with 2 ml ice-cold EZ Lysis Buffer; transfer rinsate to conical and mix gently. Incubate on ice 5 min.

11 Centrifuge at 500 × g for 5 min at 4°C.

12 Resuspend in 4 ml ice-cold EZ Lysis Buffer by gentle pipetting and incubate on ice for 5 min.

13 Centrifuge at 500 × g for 5 min at 4°C

14 Resuspend in 4 ml ice-cold Nuclei Suspension Buffer.

15 Centrifuge at 500 × g for 5 min at 4°C.

16 Resuspend in ~1 ml ice-cold Nuclei Suspension Buffer with Ruby (depending on expected yield). Filter through a 35-µm cell strainer.

17 **For FACS:**
As much as possible, the sorted cells should be collected into a small volume (<30 ul final volume for the v2 kit, <45 for v3) to avoid the need to spin down after sorting. We have found that a 100 uM nozzle leads to a larger collection volume than may be desirable (>30ul for ~7000 events); if a smaller nozzle (e.g., 85 uM) gives adequate nuclear quality, it may be preferable.

For 10x:
For the cDNA amplification step, calculate based on ~50-60% recovery and then add 1-2 cycles over the standard recommended number, as nuclei have less RNA than cells