Aug 14, 2023

BIDMC TMC - low input single nuclei sequencing isolation (NNLB)

DOI
dx.doi.org/10.17504/protocols.io.x54v9pp8qg3e/v1

Luciano G Martelotto[1], aploumak[2,3,4], Nikolaos Kalavros[2,3,4,5], Ioannis Vlachos[2,3,4,5], Shuoshuo Wang[6]

[1]University of Adelaide; [2]Spatial Technologies Unit, Beth Israel Deaconess Medical Center; [3]Department of Pathology, Beth Israel Deaconess Medical Center; [4]Harvard Medical School; [5]Broad Institute of MIT and Harvard; [6]Beth Israel Deaconess Medical Center

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

Nikolaos Kalavros
Beth Israel Deaconess Medical Center

DOI: https://dx.doi.org/10.17504/protocols.io.x54v9pp8qg3e/v1

Protocol Citation: Luciano G Martelotto, aploumak, Nikolaos Kalavros, Ioannis Vlachos, Shuoshuo Wang 2023. BIDMC TMC - low input single nuclei sequencing isolation (NNLB). **protocols.io https://dx.doi.org/10.17504/protocols.io.x54v9pp8qg3e/v1**

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

Created: August 14, 2023

Last Modified: August 14, 2023

Protocol Integer ID: 86446

Keywords: nuclei from isolated peripheral lymphatic vessel, single nuclei isolation protocol, isolated peripheral lymphatic vessel, sequencing isolation, single nuclei isolation, nuclei isolation, low input single nuclei, bidmc tmc, single nuclei, intact nuclei, low input tissue, nuclei

Funders Acknowledgements:
NIH
Grant ID: U54-165440

Abstract

Single nuclei isolation protocol used by the BIDMC TMC to isolate intact nuclei from very low input tissues. Has been used successfully by the BIDMC TMC to isolate and sequence nuclei from isolated peripheral lymphatic vessels. Developed in collaboration with Dr. Luciano Martelotto

Troubleshooting

Abstract

1 **No nuclei left behind (NNLB)**

The single-nuclei RNA sequencing protocol used by the Spatial Technologies Unit at the Beth Israel Deaconess Medical Center in order to isolate and sequence RNA from very small samples (<1000 cells). All steps are optimized to ensure maximum cell recovery.

This protocol has been successfully applied in order to perform single-nucleus RNA sequencing on isolated peripheral lymphatic vessels for HuBMAP.

This protocol has been developed in collaboration Luciano Martelotto at the University of Adelaide.

Necessary materials

2
- Very small pieces of tissue (~10-20 mg range. 1/2 - 1 grain of rice size)
- EZ Lysis Buffer (NUC101-1KT, Sigma-Aldrich)
- Pestle for 0.5 mL Eppendorf tube (Fisher Scientific, NC9719656)
- DNA LoBind 0.5 mL and 1.5 mL Eppendorf tubes
- High recovery PCR tubes
- Swinging backet centrifuge (essential!!)
- Protector RNAse Inhibitor (Sigma-Aldrich) or RiboLock RNAse Inhibitor (Thermofisher)
- pluriStrainer Mini 70 µm Cell Strainer
- pluriStrainer Mini 40 µm Cell Strainer
- Flowmi Cell Strainers

Buffers

3 **Salty-Ez10 or Salty-Ez50 Lysis Buffer**

- 10 mM Tris-HCl pH 7.5
- 146 mM NaCl
- 1 mM CaCl2
- 21 mM MgCl2
- 0.03% Tween-20 (Sigma Aldrich, P9416-50ML)
- 0.01% BSA (Miltenyi, 130-091-376)
- 1 mM DTT (Thermo)
- 10% Ez Lysis Buffer OR 50% Ez Lysis Buffer (Sigma Aldrich)
- 0.2-1 U/uL Protector RNAse Inhibitor

Wash and Resuspension Buffer

- 1x PBS
- 1% BSA (Molecular Grade)
- 0.2-1 U/uL Protector RNAse Inhibitor

Important Notes

4
- Salty-Ez10 is milder than Salty-Ez50, so choice Salty-Ez10 or Salty-Ez50 Lysis Buffer depends on the tissue and some testing will be required.
- It is important to keep the tissue on ice at all times to prevent denaturation and degradation of the nuclei.
- DNA LoBind Eppendorf tubes and Max recovery PCR tubes are essential to avoid loses.
- Swinging bucket centrifuges are critical.

Protocol

5 Place small pieces of tissue in a pre-cooled 0.5 mL Eppendorf tube. **Note**: pieces can be fresh
or snap-frozen. Briefly spin the tube to consolidate the tissue at the bottom of the tube.

6 Add enough volume of chilled SaltyEZ10 (or SaltyEZ10) buffer **just to cover the tissue**.

7 Mice using the pestle. Perform at least 10 strokes or until the tissue is fully homogenized. Then, add 200 uL more of chilled lysis buffer, and mix by pipetting 3x with a P1000 (bore tip is
optional).

8 Incubate the homogenized tissue on ice for 10’ to allow for nuclei release. Note: 10’ is usually
enough for most samples, yet it may require optimization to avoid over-lysing. I recommend
not to process more than 4 samples at the time because timing because difficult.

9 Filter homogenate using a 70 um-strainer mesh (pluriStrainer Mini 70 µm Cell Strainer) fitted
on a **pre-cooled** 1.5 uL Eppendorf. This step is to remove undigested tissue or fat prior to centrifugation. Spin at 4°C for 10” to ensure all the volume is collected. Transfer the volume
to a **pre-cooled** 0.5 mL Eppendorf tube (~350 uL). Add 100 uL of lysis buffer to the 1.5 uL
Eppendorf to collect any leftover nuclei, wash walls and transfer onto the 0.5 mL Eppendorf
tube (~450 uL).

10 Centrifuge the nuclei at 500G for 5′ at 4°C to pellet the nuclei using a **swinging bucket centrifuge**. For this you will need to make an "0.5 mL adapter" if you don't have 1.5 mL and 0.5 mL Eppendorf tubes enabled rotors. To do so, remove cap of a 5 mL and a 1.5 mL Eppendorf tubes, and insert the uncapped 1.5 mL tube inside the 5 mL tube.

11 Carefully remove the supernatant, leaving ~30 uL behind. Then resuspend the nuclei pellet by
adding 50 uL of chilled lysis buffer (~80 uL) and transfer the nuclei to a 0.2 uL PCR tube. Add
100 uL of lysis buffer to the 0.5 uL Eppendorf to collect any leftover nuclei, wash walls and
transfer onto the 0.2 mL PCR tube (~160 uL).

12 Centrifuge the nuclei at 500xg for 5′ at 4°C to pellet the nuclei using a swinging bucket centrifuge. For this you will need to make an "o.2 mL adapter" removing the cap of a 0.5 mL
Eppendorf and insert the uncapped 0.5 mL tube in the 0.5 mL adapter made (step 6).

13 **Wash 1**: Carefully remove the supernatant, leaving ~30 uL behind. Very slowly, add 150 uL of Washing and Resuspension Buffer on the walls of the tube ***without resuspending the nuclei pellet*** (~180 uL).

14 Centrifuge the nuclei at 500xg for 5′ at 4°C to pellet the nuclei using a swinging bucket centrifuge.

15 **Wash 2**: Carefully remove the supernatant, leaving ~30 uL behind. Very slowly, add 150 uL of Washing and Resuspension Buffer on the walls of the tube ***without resuspending the nuclei pellet*** (~180 uL).

16 Carefully remove the supernatant, leaving ~50 uL behind and resuspend the pellet by gently pipetting 15x.

17 Count nuclei by using 5 uL of nuclei suspension and mixed 5 uL of Washing and Resuspension
Buffer (this is ½ dilution). If the nuclei prep is clumpy, add 100 uL Washing and Resuspension
Buffer and pass it through a 40 um Flowmi filter, then centrifuge 500xg for 5′ at 4°C, carefully remove the supernatant, leaving behind 50 uL. Resuspend by gently pipetting 15x (**Note: this
step can cause significant loss**).

18 Count and proceed to downstream single nuclei profiling.