Dec 13, 2024

JAX-Sen: Nuclei prep from frozen heart tissue for single-nuclei RNA-seq

DOI
dx.doi.org/10.17504/protocols.io.kxygxwy6zv8j/v1

Sandra L Daigle[1], Greggory A Perry[1], William F Flynn[2], Elise T Courtois[2]

[1]Single Cell Biology Lab, The Jackson Laboratory, USA; [2]The Jackson Laboratory for Genomic Medicine

Cellular Senescence Net...

Harshpreet Chandok
The Jackson Laboratory

DOI: https://dx.doi.org/10.17504/protocols.io.kxygxwy6zv8j/v1

Protocol Citation: Sandra L Daigle, Greggory A Perry, William F Flynn, Elise T Courtois 2024. JAX-Sen: Nuclei prep from frozen heart tissue for single-nuclei RNA-seq. **protocols.io https://dx.doi.org/10.17504/protocols.io.kxygxwy6zv8j/v1**

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

Created: December 12, 2024

Last Modified: December 13, 2024

Protocol Integer ID: 115132

Keywords: nuclei prep from frozen heart tissue, nuclei rna, rna, frozen heart tissue, nuclei, mouse heart, senescence, tissue

Funders Acknowledgements:
National Institute on Aging (NIA) JAX-Sen Senescence Tissue Mapping Center Grant
Grant ID: U54 AG079753

Abstract

We aim to study and characterize senescence in the C57BL/6J mouse heart. This protocol describes the nuclei prep from frozen heart tissue for single-nuclei RNA-seq.

Troubleshooting

Abstract

1 We aim to study and characterize senescence in the C57BL/6J mouse heart. This protocol describes the nuclei prep from frozen heart tissue for single-nuclei RNA-seq.

Reagents and Materials

2 **Nuclei Isolation**

- Chromium Nuclei Isolation with RNase Inhibitor Kit: 1000494 (10x Genomics)
- MACS BSA Stock Solution: 130-091-376 (Miltenyi)
- DPBS: 14190144 (Thermo Fisher Scientific)
- Eppendorf™ DNA LoBind1.5 mL Microcentrifuge Tubes: 13-698-791 (Thomas Scientific)
- Lobind Eppendorf 2.0mL tubes: 13-698-792 (Thermo Fisher Scientific)
- BOApluriStrainer Mini 70 µm: 43-10070-50 (pluriSelect)
- BOApluriStrainer Mini 40 µm: 43-10040-50 (pluriSelect)
- Anti-Nucleus Microbeads: 130-132-997 (Miltenyi)
- LS Columns: 130-042-401 (Miltenyi)
- QuadroMACS Separator: 130-090-976 (Miltenyi)
- MultiStand: 130-042-303 (Miltenyi)

Cell Counting: LUNA FX7

- Acridine, Orange/Propidium Iodide Stain: F23001(New England BioGroup, LLC)
- Luna Slides:
- Ultra-low Fluorescent Slides: Fluorescent and Brightfield Mode (New England BioGroup, LLC)

Procedure

3 **Before Starting:**

3.1 Pre-cool centrifuge, buffers, and consumables with sample contact (columns, tubes and Strainers, etc.) at 4 °C.

3.2 Prepare Buffers

3.3 1 Heart tissue-tissue cut in quarters before snap freezing-each quarter per column

3.4 4 columns per 1 Heart x 2 hearts per batch (8 total columns)

3.5

10X Chromium Nuclei Isolation Lysis Buffer							
Item	**Stock**	**Final**	**1X**	**2X**	**4X**	**6X**	**8X**
Lysis Buffer-µL	n/a	n/a	550	1,100	2,200	3,300	4,400
Reducing Agent B-µL	n/a	n/a	0.55	1.1	2.2	3.3	4.4
Surfactant A-µL	n/a	n/a	5.5	11.0	22.0	33.0	44.0
Total			556.05	1,112.1	2,224.2	3,336.3	4,448.4
10X Chromium Debris Removal							
Item	**Stock**	**Final**	**1X**	**2X**	**4X**	**6X**	**8X**
Debris Removal-µL	n/a	n/a	550	1,100	2,200	3,300	4,400
Reducing Agent B-µL	n/a	n/a	0.55	1.1	2.2	3.3	4.4
Total			551	1,101.1	2,202.2	3,303.3	4,404.4
Wash Buffer (WB)-for 2x wash							
Item	**Stock**	**Final**	**1X**	**2X**	**4X**	**6X**	**8X**
PBS pH 7.2-µL	1X	-	1,587	3,174	6,348	9,522	12,696
BSA-µL	10%	2%	400	800	1,200	2,400	3,200
RNAse Inhibitor-µL	40U/µL	.25U/µL	13	25	38	75	100
Total-µL			2,000	4,000	6,000	12,000	16,000
Resuspension Buffer (NRB)-for final resuspension							
Item	**Stock**	**Final**	**1X**	**2X**	**4X**	**6X**	**8X**
PBS pH 7.2-µL	1X	-	116	233	465	698	930
BSA-µL	10%	2%	30	60	120	180	240
RNAse Inhibitor-µL	40U/µL	1U/µL	4	8	15	23	30
Total-µL			150	300	600	900	1,200

4 Chromium Procedure for Tissue Lysis and nuclei extraction

4.1 Transfer frozen tissue (1/4 heart) to pre-chilled 1.5mL tube on ice

4.2 Add 200µL Lysis Buffer and dissociate the tissue using plastic pestle, leave on ice. Continue with the rest of the samples in the batch

4.3 Add additional 300µL Lysis Buffer. Pipette mix 10x, if the tip clogs do additional pestling Incubate on ice for 15min

4.4 Pipette lysate into pre-chilled 10x columns (2mL tube)

4.5 Centrifuge (use fixed rotor) 1600 rcf 20 sec 4C

4.6 Discard column, mix well and Spin again 500 rcf 3 min 4C (swing bucket)

4.7 Remove supernatant - leave behind ~200µL if pellet in not visible

5 **Debris Removal Steps**

5.1 Resuspend pellet in 500µL Debris Removal Buffer, gently mix 15x

5.2 Spin 700 rcf 10 min 4C (swing bucket)

5.3 Remove supernatant - leave behind ~200µL if pellet in not visible

5.4 Resuspend pellet in 1mL Wash Buffer (WB), gently mix 15x

5.5 Spin 500 rcf 5 min 4C (swing bucket)

5.6 Remove supernatant - leave behind ~200µL if pellet in not visible

5.7 Resuspend pellet in 1mL Wash Buffer (WB), gently mix 15x

5.8 Spin 500 rcf 5 min 4C (swing bucket)

5.9 Resuspend pellet in 40-60µL (NRB), gently mix 15x combine nuclei from all 4 tissues from 1 heart

Optional: use 40µm if samples are clumpy

Count