Jul 27, 2023

WU sn-prep Protocol for solid tumors- joint snRNA+ATAC v2.9

Nature Cancer

DOI
dx.doi.org/10.17504/protocols.io.261gednx7v47/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.261gednx7v47/v1

External link: https://doi.org/10.1038/s43018-024-00773-6

Protocol Citation: Reyka Jayasinghe, Wagma Caravan, Andrew Houston, Nataly Naser Al Deen 2023. WU sn-prep Protocol for solid tumors- joint snRNA+ATAC v2.9. **protocols.io https://dx.doi.org/10.17504/protocols.io.261gednx7v47/v1**

Manuscript citation:
Michael D. Iglesia, Reyka G. Jayasinghe, Siqi Chen, Nadezhda V. Terekhanova, John M. Herndon, Erik Storrs, Alla Karpova, Daniel Cui Zhou, Nataly Naser Al Deen, Andrew T. Shinkle, Rita Jui-Hsien Lu, Wagma Caravan, Andrew Houston, Yanyan Zhao, Kazuhito Sato, Preet Lal, Cherease Street, Fernanda Martins Rodrigues, Austin N. Southard-Smith, André Luiz N. Targino da Costa, Houxiang Zhu, Chia-Kuei Mo, Lisa Crowson, Robert S. Fulton, Matthew A. Wyczalkowski, Catrina C. Fronick, Lucinda A. Fulton, Hua Sun, Sherri R. Davies, Elizabeth L. Appelbaum, Sara E. Chasnoff, Madelyn Carmody, Candace Brooks, Ruiyang Liu, Michael C. Wendl, Clara Oh, Diane Bender, Carlos Cruchaga, Oscar Harari, Andrea Bredemeyer, Kory Lavine, Ron Bose, Julie Margenthaler, Jason M. Held, Samuel Achilefu, Foluso Ademuyiwa, Rebecca Aft, Cynthia Ma, Graham A. Colditz, Tao Ju, Stephen T. Oh, James Fitzpatrick, E. Shelley Hwang, Kooresh I. Shoghi, Milan G. Chheda, Deborah J. Veis, Feng Chen, Ryan C. Fields, William E. Gillanders, Li Ding (2024) Differential chromatin accessibility and transcriptional dynamics define breast cancer subtypes and their lineages.Nature Cancer doi: 10.1038/s43018-024-00773-6

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

Created: July 26, 2023

Last Modified: July 27, 2023

Protocol Integer ID: 85530

Keywords: prep protocol for solid tumor, snrna, wu sn, chromatin accessibility, nuclei, snatac, genetic expression

Abstract

Nuclei dissociation protocol adapted from WU sn-prep Protocol for Solid Tumors - snRNA protocol v2.8 for simultaneous profiling of genetic expression (snRNA) and chromatin accessibility (snATAC)

Troubleshooting

Reagents and Tools

1 1x Lysis buffer (2mL):
10mM Tris-HCl (pH 7.4) (Thermo; 15567027), 20µL
10mM NaCl (Thermo; AM9759), 4µL
3 mM MgCl2 (Thermo; AM9530G), 6µL
NP-40 substitute (Sigma, 74385-1L), 2µL
1 M DTT (Sigma, 646563), 2µL
Nuclease Free Water (Invitrogen, AM9937), 1.966mL

2 Lysis Dilution Buffer (10mL):
10mM Tris-HCl (pH 7.4) (Thermo; 15567027), 100µL
10mM NaCl (Thermo; AM9759), 20µL
3 mM MgCl2 (Thermo; AM9530G), 30µL
1 M DTT (Sigma, 646563), 10µL
Nuclease Free Water (Invitrogen, AM9937), 9.840mL

3 0.1x Lysis Buffer (10mL):
1x Lysis Buffer (1mL) + Lysis Dilution Buffer (9mL)

4 Wash and Resuspension buffer (WR buffer, 10mL):
1X PBS, 8mL
10% Stock BSA Solution (MACS, 130-091-376), 2mL

5 Roche Protector RNase inhibitor-2000 U/µL (Millipore Sigma 3335399001)

6 Trypan blue (2X) - filtered at 0.22µm

7 7-AAD (7-Aminoactinomycin D) (Millipore Sigma SML1633-1ML)

8 Glass homogenizers (Fisher: 2mL tube- K8853030002, Small clearance pestle- K8853020002, Large clearance pestle- K8853010002)

9 Fine forceps and scalpels

General Notes

10
- Keep everything on ice (or in the cold room)
- Use RNase free reagents and consumables, before starting wipe down all surfaces/pipettes with RNase away and 70% ethanol
- Avoid foam and bubbles as much as possible with gentle strokes and pipetting slowly

Nuclei Dissociation

11 If using frozen tissue sample, use a scalpel (aided by a pair of fine forceps) to cut the cold samples (25-35mg) into 2mm pieces, add 1 ml of ice-cold 0.1X lysis buffer and 30 µL RNase Inhibitor, load into the glass homogenizer. Homogenize with 4-6 push/pulls using the pestel, incubate on ice for 1 min with an additional 500-1000µL of 0.1X cold lysis buffer. Pipette gently for 4 times. Incubate on ice again for up to 1 min.

11.1 If using pulverized powder, start with 15-35 mg total, add 1 ml of ice-cold 0.1X lysis buffer and 30 µL RNase Inhibitor, pipette the powder/lysis buffer mix gently for 6-8 times. Let sit on ice for 30". Pipette another 4-6 times with an additional 500-1000µL of 0.1X cold lysis buffer. Incubate on ice again for 1' – could be reduced (to like 20-45"). Add to the glass homogenizer, use the pestle for 3-4 push/pulls.

11.2 If using OCT sections, start with 300-450µm total sectioned into a 1.5mL tube. Add 1 ml of ice-cold 0.1X lysis buffer and 30 µL RNase Inhibitor, pipette the mix gently for 10-12 times, will be sticky as the OCT thaws. Let sit on ice for 30". Pipette another 4-6 times with an additional 500-1000µL of 0.1X cold lysis buffer. Incubate on ice again for 1' – could be reduced (to like 20-45"). Add to the glass homogenizer, dounce with the pestle for 6-8 push/pulls.

12 Filter the homogenate through a 40µM cell strainer on ice on top of a 50ml conical tube. Wash the filter with 1ml WR buffer. Collect this into the same tube, the total filtrate is ~3 ml.
If there is still tissue on the strainer, backwash with 2 mL 0.1x Lysis buffer, follow previous steps again to dissociate completely. If going to FACS with the backwash, proceed with this sample as if it were a different tissue but sort into same collection tube.

13 Transfer the filtrate to a 5ml Eppendorf tube. Centrifuge at 500g for 6' at 4°C, resuspend with 100-400µL WR buffer (depending on pellet size) and 10 µL RNase inhibitor.

14 Transfer into a FACS sorting tube and add 3µL 7-AAD per 500 µL of resuspended sample, incubate for 10 minutes before sorting at FACS. After resuspending sample in wash buffer, if small chunks are still visible (after ~3 minutes of resuspension) use 40µM mini-strainer over FACS tube to remove chunks (proceeding to FACS with sample in current condition will clog machine and will result in additional lost sample).

15 Add 50µL 10% BSA solution and 10µL RNase inhibitor and into a 2mL nonbinding tube for collection. Sort 300-400K of the nuclei into the collection tube.

16 After FACS sorting is done centrifuge the 2ml collection tube at 500g for 6′ at 4°C. There will not be a visible pellet, remove all the supernatant (likely ~3µL will be left).

17 Resuspend in 3µL 2x Nuclei Dilution Buffer (prepared as directed by 10x Genomics, pg. 28) and 1µL RNase inhibitor, final volume should be ~10µL.

18 Quantify nuclei quality and quantity using a hemacytometer or Countess II utilizing Trypan blue.

19 Load desired concentration of nuclei, proceed with the protocol as outlined by 10x Genomics which can be found **here**.