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10X Genomics Single-Nucleus Multiome (RNA + ATAC) Assay for Profiling Adult Human Tissues V.2

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Human Cell Atlas Method Development Community KPMP 1

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10X Genomics Single Cell 3' (v3) RNA sequencing is a microdroplet-based method that permits the effective capture and sequencing of the mRNA and pre-mRNA molecules from single nuclei [1]. RNA molecules are transcribed and processed within the nucleus before exporting to ER for translation into proteins. As such, nuclear RNA is a mixture of nascent transcripts, partially or fully processed mRNA, and various non-coding RNA molecules. The total RNA content within the nucleus is roughly 10% of the RNA content in a whole cell, but has been found to accurately represent whole cell expression values in adult human tissues [2,3] including the kidney [4]. 10x Genomics Single Cell ATAC sequencing is a microdroplet-based method that allows for the effective capture, sequencing and profiling of accessible chromatin in single nuclei. Chromatin accessibility is a major determinant of gene regulation, defining the transcriptional regulatory networks that determine cellular identity and function as well as additional biological processes (e.g., differentiation, proliferation, development and responses to the extracellular environment). The 10X Multiome ATAC + Gene Expression assay permits capture of both RNA expression and epigenomic profiles from the same nuclei for a deeper understanding of cell type or state gene regulation. Here we present a modified version of the published 10X protocol [5] that we have adapted for the processing of nuclei isolated from adult human organs or tissues (e.g. kidney).

Nuclei can be readily isolated from frozen tissues with a combination of chemical and physical treatments that can effectively circumvent the non-uniform or incomplete dissociation of solid tissues into single cells, as well as RNA degradation or artefacts (such as stress response) during dissociation. This 10X Multiome ATAC + Gene Expression protocol permits the generation of a molecular atlas of a human organ or tissue with comprehensive cell types and minimal processing artifacts.

References

1. Chromium Single Cell 3' Reagent Kits v3 User Guide (Rev A) CG000183, support.10xgenomics.com.
2. Lake et al. (2016). Science, doi:10.1126/science.aaf1204.
3. Lake et al. (2018). Nature Biotechnology, doi:10.1038/nbt.4038.
4. Lake et al. (2019). Nature Communications, doi:10.1038/s41467-019-10861-2.
5. Chromium Next GEM Single Cell Multiome ATAC + Gene Expression (CG000338 Rev D).

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http://genome-tech.ucsd.edu/ZhangLab/

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Kidney, KPMP, Single Nucleus, RNA Sequencing, 10X Genomics, Chromatin Accessibility, ATAC

protocol ,

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Full protocol is from 10X Genomics. All modifications are to the original protocol (Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (Rev D) CG000338, support.10xgenomics.com)

MATERIALS

Chromium Next GEM Single Cell Multiome ATAC Gene Expression **10x Genomics Catalog #PN-1000283**

Chromium Next GEM Chip J Single Cell Kit **10x Genomics Catalog #PN-1000234**

Single Index Kit N Set A **10x Genomics Catalog #1000212**

Dual Index Kit TT Set A **10x Genomics Catalog #1000215**

Nuclease-free water
Ethanol (200 proof)
SPRIselect reagent set
50% glycerol
10% Tween 20
Low TE Buffer (10mM Tris-HCl, pH 8.0, 0.1 EDTA)
Qiagen buffer EB
Qubit dsDNA HS Assay Kit
PCR strip tubes with cap
LoBind 1.5 ml tubes
Qubit Assay tubes

Isolate Nuclei

1 Prepare nuclei according to the protocol "Isolation of single nuclei from solid tissues" steps 1-14, with the following modifications:
- Tissue sections are cut and stored on dry ice until processed for nuclei isolation (locally or shipped/stored overnight). Sections **cannot** be stored in RNAlater or in a -80C freezer.
- Step 1: replace Enzymatics RNAse Inhibitor with Protector RNase Inhibitor (Sigma-Aldrich, Catalog #3335402001), increase concentration of RNAse Inhibitor from 0.04 U/µl to 1.0 U/µl, add cOmplete protease inhibitor cocktail (Roche, cat #11836153001) for final 1X concentration and exclude DAPI.
- Step 4: To PBSE buffer, add 1 tablet of cOmplete protease inhibitor cocktail to 50 µl PBSE.
- Step 13: Determine total amount of wash buffer needed and add 0.1% Protector RNase Inhibitor (Sigma, PN-3335399001) to volume of PBSE + cOmplete protease inhibitor.

2 Resuspend nuclei in **30 µL** to **100 µL** of 1X Diluted Nuclei Buffer (20X nuclei buffer, PN2000207, provided by 10x Genomics), volume depends on target concentration. 1X Diluted Nuclei Buffer is prepared following the user guide Chromium Next GEM Single Cell Multiome ATAC + Gene Expression (CG000338 Rev D).

3 Count nuclei (e.g. BioRad T20 Cell Counter) .

4 Check nuclei integrity under fluorescent microscope using DAPI channel. Nuclei should appear distinct, have rounded borders and the majority occurring as singlets. High clumping rates would indicate damaged nuclei and would require re-filtering using 30-µm CellTrics filter or exclusion.

5 Dilute nuclei stock to be in the 1,000-3,200 nuclei per µL range in **5 µL** total volume using the dilution guide

QC cutoff: minimum of 5,000 nuclei

Transposition

6 Prepare Reagents for use
1. Thaw, vortex, and centrifuge ATAC Buffer B.
2. Centrifuge ATAC Enzyme B (maintain **On ice**) before adding to Transposition Mix.

7 Prepare Transposition Mix.
1. Prepare **On ice** . Pipette mix 10X and centrifuge briefly.
2. Add **10 µL** Transposition Mix to each tube of a PCR 8-tube strip **On ice** .
3. Calculate the volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of **5 µL** using the Nuclei Concentration Guidelines.
4. Add the calculated volume of Diluted Nuclei Buffer to the Transposition Mix, pipette mix, and centrifuge briefly.

Citation: Kimberly Conklin, Bo Zhang, Amanda Knoten, Dinh Diep, Blue Lake, Sanjay Jain, Kun Zhang 10X Genomics Single-Nucleus Multiome (RNA + ATAC) Assay for Profiling Adult Human Tissues https://dx.doi.org/10.17504/protocols.io.5qpvoby69l4o/v2

5. Gently pipette mix the Nuclei Stock before adding the calculated volume of the Nuclei Stock to the tube containing the Transposition Mix. Gently pipette mix 6X (pipette set to 10 µl). DO NOT centrifuge.

8 Isothermal Incubation 1h

1. Incubate at **37 °C** for **01:00:00** in a thermal cycler with lid set at **50 °C** for transposition and addition of adapter sequences, which are added to the ends of the DNA fragments.
2. Proceed immediately to next step.

GEM Generation and Barcoding

9 Prepare Reagents for use

1. Equilibrate gel beads to **Room temperature** for **00:30:00** .
2. Thaw, vortex, and centrifuge Barcoding Reagent Mix, Template Switch Oligo, and Reducing Agent B. Verify no precipitate in Reducing Agent B.
3. Centrifuge Barcoding Enzyme Mix (maintain **On ice**) before adding to Master Mix.

10 Prepare Master Mix

1. Prepare **On ice** . Pipette mix 10X and centrifuge briefly.
2. Keep **On ice** .
3. Add **60.0 µL** Master Mix to each tube containing Transposed Nuclei **On ice** .

11 Load Chromium Single Cell J Chip 1m 5s

1. Assemble Chromium Chip J in a 10X Chip Holder.
2. Must load chip in order according to row label for microfluidic channels to work properly: Row 1 → Row 2 → Row 3
3. Make sure NO bubbles are introduced while loading chip.
4. Dispense 50% glycerol solution into unused Chip Wells (if <8 samples used per chip)
   a. **70 µL** into unused wells in Row 1
   b. **50 µL** into unused wells in Row 2
   c. **45 µL** into unused wells in Row 3
5. Add **60.0 µL** Master Mix to each tube containing Transposed Nuclei on ice. Gently pipette mix 5X.
6. Load **70 µL** Master Mix and Transposed Nuclei into the bottom center of each well in Row 1 without introducing bubbles.
7. Snap the Gel Bead strip into a 10x Vortex Adapter. Vortex **00:00:30** . Remove the Gel Bead strip and centrifuge for ~ **00:00:05** . Confirm there are no bubbles at the bottom of the tubes and liquid levels look even.
8. Load **50 µL** Gel Beads in Row 2. Dispense slowly and without introducing bubbles. Wait **00:00:30** .

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9. Load **40 µL** Partitioning Oil into wells of Row 3. Proceed immediately to the next step (and to running Chip in Chromium Controller).
10. Attach 10X gasket. Align the top-notch. Ensure gasket holes are aligned with the wells. Avoid touching the smooth gasket surface. Do not press down on the gasket.

12 Run the Chromium Controller 18m

1. Press the eject button on the Controller to eject the tray.
2. Place the assembled chip with the gasket in the tray. Press the button to retract the tray.
3. Confirm the program on screen. Press the play button.
4. At completion of the run (~ **00:18:00**), the Controller will chime. Proceed immediately to the next step.

Firmware Version 4.00 or higher is required in the Chromium Controller for use of this protocol.

13 Transfer GEMs

1. Chill a PCR 8-tube strip **On ice** .
2. Press the eject button to remove the Chip.
3. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.
4. Check the volume in rows 1-3. Abnormally high volume in any well indicates a clog.
5. Slowly aspirate 100 µL GEMs from the lowest points of the Recovery Wells without creating a seal between the pipette tips and the wells.

GEMs should appear opaque and uniform across all channels. Excess partitioning oil (clear) in the pipette tips indicates a potential clog.

6. Slowly dispense (~ **00:00:20**) GEMs into the tube strip **On ice** with the pipette tips against the sidewalls of the wells.

14 GEM Incubation 1h 15m

1. Incubate in a thermocycler for **01:15:00** to complete reverse transcription.
2. During GEM incubation, equilibrate Quenching Agent to **Room temperature** .
3. Upon completion of GEM incubation, proceed immediately to next step.

15 Quenching Reaction

1. Add 5 µl Quenching Agent to each sample to stop the reaction.
2. Slowly pipette mix 10X (pipette set to 90 µl). The solution will be viscous, ensure that no liquid remains along the the sidewalls of the tube or the pipette tips.
3. Store at **-80 °C** for up to 4 weeks or proceed to the next step.

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16 Post GEM Incubation Cleanup -- prepare reagents for use

1. Thaw, vortex, and centrifuge a tube of Reducing Agent B.
2. Thaw Cleanup Buffer at **65 °C** for **00:10:00** with shaking at max rpm on a thermomixer, then cool to room temperature before use. Verify there are no visible crystals.

12m

17 Post GEM Incubation Cleanup -- Dynabeads

1. Add **125 µL** Recovery Agent to each sample at room temperature. DO NOT pipette mix or vortex. Gently invert tube 10X to mix and centrifuge briefly.
2. Carefully aspirate **125 µL** of pink oil phase from the bottom of the tube. DO NOT aspirate any aqueous sample.
3. Prepare Dynabeads Cleanup Mix (follow recipe in User Guide).
4. Vortex and add **200 µL** Dynabeads Cleanup Mix to each sample. Pipette 10X to mix (pipette set to 200 µl).
5. Incubate at room temperature for **00:10:00** with caps open.
6. Prepare Elution Solution I (follow recipe in User Guide). Vortex and centrifuge briefly.
7. After the 10-minute incubation period, place the samples on a magnetic separator. Wait until solution clears before removing the supernatant.
8. Add **300 µL** of freshly prepared 80% ethanol. Wait **00:00:30** before removing ethanol.
9. Add **200 µL** of freshly prepared 80% ethanol. Wait **00:00:30** before removing ethanol.
10. Remove from magnet and immediately add **50 µL** Elution Solution 1 to avoid clumping. Pipette mix (pipette set to 50 µl) without introducing bubbles.
11. Incubate **00:01:00** at **Room temperature** .
12. Centrifuge briefly and move back to magnet.
13. After solution clears, transfer **50 µL** to a new tube strip.

7m 30s

18 Post GEM Incubation Cleanup -- SPRIselect

1. Add **90 µL** SPRIselect reagent to each sample and pipette mix thoroughly.
2. Incubate **00:05:00** at **Room temperature** .
3. Centrifuge briefly and place on magnet until solution clears. Remove supernatant.
4. Add **200 µL** freshly prepared 80% ethanol to pellet and wait **00:00:30** before removing ethanol.
5. Repeat step 4 for a total of 2 washes.
6. Centrifuge briefly and place on magnet.
7. Remove any remaining ethanol (note: residual ethanol can inhibit Pre-Amplification PCR and impact assay performance).
8. Remove from magnet and immediately add **46.5 µL** Buffer EB. Pipette mix (pipette set to 45 µl) without introducing bubbles.
9. Incubate **00:02:00** at **Room temperature** .
10. Centrifuge briefly and return to magnet.
11. After solution clears, transfer **46 µL** to a new tube strip.

Pre-Amplification PCR & Cleanup

18h 30m

19 Pre-Amplification PCR

1. Thaw, vortex, and centrifuge Pre-Amp Primers.
2. Centrifuge Pre-Amp Mix (maintain **On ice**) before adding.
3. Prepare Pre-Amplification Mix **On ice** (follow recipe in User Guide). Pipette mix 10X and centrifuge

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briefly.

4. Add **54 µL** Pre-Amplification Mix to each sample. Pipette mix and centrifuge briefly.
5. Incubate in a thermal cycler (run time: **00:30:00**).
6. Store at **4 °C** for up to **18:00:00** or proceed to the next step.

20 Pre-Amplification Cleaup -- SPRIselect

3d 0h 8m

1. Add **160 µL** SPRIselect reagent to each sample and pipette mix thoroughly.
2. Incubate for **00:05:00** at **Room temperature** .
3. Centrifuge briefly and place on magnet.
4. Once solution is clear, remove supernatant.
5. Add **300 µL** freshly prepared 80% ethanol. Wait **00:00:30** before removing the ethanol.
6. Add **200 µL** 80% ethanol. Wait **00:00:30** before removing the ethanol.
7. Centrifuge briefly and place on magnet. Remove any remaining ethanol.
8. Remove from magnet and immediately add **160.5 µL** Buffer EB. Pipette mix (pipette set to 150 µl) without introducing bubbles.
9. Incubate for **00:02:00** at **Room temperature** .
10. Centrifuge briefly and return to magnet.
11. After solution clears, transfer **160 µL** to a new tube strip.
12. Store at **4 °C** for up to **72:00:00** or at **-20 °C** for long-term storage or proceed to the next step.

21 Pre-Amplified, SPRI-cleaned Sample Split

- Move **40 µL** to a new tube strip for ATAC Library Construction.
- Move **35 µL** to a new tube strip for cDNA Amplification.
- Store the remaining **85 µL** pre-amplified, SPRI-cleaned product at **-20 °C** long term for generating additional libraries.

ATAC Library Construction

22 ATAC Sample Index PCR

3d 0h 30m

1. Bring Sample Index Plate N, Set A to **Room temperature** .
2. Thaw, vortex, and centrifuge a tube of SI-Primer B (maintain **On ice**).
3. Centrifuge Amp Mix (maintain **On ice**) before adding.
4. Prepare Sample Index PCR Mix (follow recipe in User Guide).
5. Add **57.5 µL** Sample Index PCR Mix to the **40 µL** aliquot of pre-amplified sample. Pipette mix and centrifuge briefly.
6. Add **2.5 µL** of an individual Sample Index N, Set A to each sample. Pipette mix and centrifuge briefly.
7. Incubate in a thermal cycler to amplify DNA (run time: approximately **00:30:00**).

- Use Cycle Number Optimization Table (in User Guide) for total number of cycles (based on Targeted Nuclei Recovery).
- For Targeted Nuclei Recovery of 6,001-10,000 nuclei, perform 7 cycles.

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8. Store at **4 °C** for up to **72:00:00** or proceed to the next step.

23 ATAC Post Sample Index Double Sided Size Selection -- SPRIselect

3d 0h 13m

1. Add **60 µL** SPRIselect reagent (0.6X) to each sample. Pipette mix.
2. Incubate for **00:05:00** at **Room temperature** .
3. Place on magnet until solution clears.
4. Transfer **150 µL** supernatant to a new tube strip. DO NOT discard the supernatant.
5. Add **95 µL** SPRIselect reagent (1.55X) to each sample (supernatant). Pipette mix.
6. Incubate for **00:05:00** at **Room temperature** .
7. Place on magnet until solution clears and remove the supernatant.
8. Add **300 µL** freshly prepared 80% ethanol to the pellet. Wait **00:00:30** before removing ethanol.
9. Add **200 µL** 80% ethanol to the pellet. Wait **00:00:30** before removing ethanol.
10. Centrifuge briefly before returning to magnet and removing any residual ethanol.
11. Remove from the magnet and immediately add **20.5 µL** Buffer EB. Pipette mix.
12. Incubate for **00:02:00** at **Room temperature** .
13. Centrifuge briefly and place on magnet.
14. Once solution clears, transfer **20 µL** to a new tube strip.
15. Store at **4 °C** for up to **72:00:00** or at **-20 °C** for long-term storage.

Post Library Construction QC:
- Quantify library (e.g. using Qubit dsDNA HS assay).
- Estimate library size range (e.g. using TapeStation HS D or Bioanalyzer) - expected size range is 170-700 bp, including nucleosome pattern of at least 3 different bands/peaks.

cDNA Amplification PCR & Cleanup

24 cDNA Amplification

3d

1. Thaw, vortex, and centrifuge cDNA Primers.
2. Centrifuge Amp Mix (maintain **On ice**) before adding.
3. Prepare cDNA Amplification Mix **On ice** (follow recipe in User Guide). Vortex and centrifuge briefly.
4. Add **65 µL** cDNA Amplification Reaction Mix to **35 µL** pre-amplified sample.
5. Pipette mix 15X (pipette set to 90 µl) and centrifuge briefly.
6. Incubate in a thermocycler to amplify cDNA (run time: approximately 30-40 minutes).

- Use Cycle Number Optimization Table (in User Guide) for total number of cycles (based on Targeted Nuclei Recovery).
- For Targeted Nuclei Recovery >6,000, perform 6 cycles.

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7. Store at **4 °C** for up to **72:00:00** or at **-20 °C** for up to 4 weeks, or proceed to the next step.

25 cDNA Cleanup -- SPRIselect 3d 0h 9m 30s

1. Add **60 µL** SPRIselect reagent (0.6X) to each sample and pipette mix 15X (pipette set to 150 µl).
2. Incubate for **00:05:00** at **Room temperature** .
3. Place on magnet until solution clears. Remove supernatant
4. Add **200 µL** freshly prepared 80% ethanol to the pellet. Wait **00:00:30** before removing ethanol.
5. Repeat step 4 for a total of 2 washes.
6. Centrifuge briefly before returning to magnet and removing any residual ethanol.
7. Air dry for **00:02:00** (DO NOT exceed 2 minutes as this will decrease elution efficiency).
8. Remove from the magnet and elute with **40.5 µL** Qiagen Buffer EB. Pipette mix 15X.
9. Incubate for **00:02:00** at **Room temperature** .
10. Place the tube strip on the magnet until the solution clears.
11. Transfer **40 µL** to a new strip tube.
12. Store at **4 °C** for up to **72:00:00** or at **-20 °C** for up to 4 weeks, or proceed to the next step.

26 cDNA QC & Quantification

1. Quantify library (e.g. using Qubit dsDNA HS assay).
2. Run TapeStation HS D5000 to obtain concentration and size
3. Expected size range for amplified cDNA: 400 to 2500 bp
4. cDNA total yield range: 80 to 1000 ng

QC Cutoff: minimum cDNA total yield of 20 ng.

3' Gene Expression Library Construction

27 Prepare Reagents for use

1. Pre-cool thermal cycler block to **4 °C** prior to preparing the Fragmentation Mix.
2. Thaw, vortex, and centrifuge Fragmentation Buffer, Adaptor Oligos, Ligation Buffer, SI Primer.
3. Maintain **On ice** Fragmentation Enzyme, DNA Ligase, and Amp Mix.
4. Bring Sample Index Plate TT, Set A to **Room temperature** .

28 Fragmentation, End Repair & A-tailing 35m

1. 1. Prepare Fragmentation Mix **On ice** (follow recipe in User Guide). Pipette mix and centrifuge briefly.
2. Transfer **10 µL** purified cDNA sample to a new tube strip **On ice** .

Note: 10 µl (25%) cDNA sample is sufficient for generating 3' Gene Expression Library. The remaining 30 µl (75%) cDNA sample can be stored at **4 °C** for up to **72:00:00** or at **-20 °C** for up to 4 weeks for generating additional 3' Gene Expression Libraries.

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3. Add **25 µL** Buffer EB to each sample.
4. Add **15 µL** Fragmentation Mix to each sample.
5. Pipette mix 15X (pipette set to 35 µl) **On ice** and centrifuge briefly.
6. Transfer to pre-cooled block and "SKIP" hold step to initiate the thermal cycler protocol (run time: approximately **00:35:00**).

29 Post Fragmentation, End Repair & A-tailing Double Sided Size Selection -- SPRIselect

3d 0h 14m 30s

1. Add **30 µL** SPRIselect reagent (0.6X) to each sample and pipette mix 15x (pipette set to 75 µl).
2. Incubate for **00:05:00** at **Room temperature** .
3. Place on magnet and wait until the solution clears. DO NOT discard supernatant.
4. Transfer **75 µL** supernatant to a new tube strip.
5. Add **10 µL** SPRIselect reagent (0.8X) to each sample and pipette mix 15x (pipette set to 80 µl).
6. Incubate for **00:05:00** at **Room temperature** .
7. Place on magnet until the solution clears.
8. Remove supernatant. DO NOT discard any beads.
9. Add **125 µL** freshly prepared 80% ethanol to the pellet. Wait **00:00:30** before removing ethanol.
10. Repeat step 9 for a total of 2 washes.
11. Centrifuge briefly before returning to magnet and removing any residual ethanol.
12. Air dry for **00:02:00** (DO NOT exceed 2 minutes as this will decrease elution efficiency).
13. Remove from the magnet and elute with **50.5 µL** Buffer EB. Pipette mix 15X.
14. Incubate for **00:02:00** at **Room temperature** .
15. Place the tube strip on the magnet until the solution clears.
16. Transfer **50 µL** to a new strip tube.
17. Store at **4 °C** for up to **72:00:00** or at **-20 °C** for up to 4 weeks, or proceed to the next step.

30 Adaptor Ligation

15m

1. Prepare Adaptor Ligation Mix **On ice** (follow recipe in User Guide). Pipette mix and centrifuge briefly.
2. Add **50 µL** Adaptor Ligation Mix to **50 µL** sample. Pipette mix 15X (pipette set to 90 µl). Centrifuge briefly.
3. Incubate in a thermal cycler to ligate adaptor (run time: **00:15:00**).

31 Post Ligation Cleanup -- SPRIselect

9m 30s

1. Add **80 µL** SPRIselect reagent (0.8X) to each sample. Pipette mix 15X (pipette set to 150 µl).
2. Incubate for **00:05:00** at **Room temperature** .
3. Place on magnet and wait until the solution clears. Remove supernatant.
4. Add **200 µL** freshly prepared 80% ethanol to the pellet. Wait **00:00:30** before removing ethanol.
5. Repeat step 4 for a total of 2 washes.
6. Centrifuge briefly before returning to magnet and removing any residual ethanol.
7. Air dry for **00:02:00** (DO NOT exceed 2 minutes as this will decrease elution efficiency).
8. Remove from the magnet and elute with **30.5 µL** Buffer EB. Pipette mix 15X.

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9. Incubate for **00:02:00** at **Room temperature** .
10. Place the tube strip on the magnet until the solution clears.
11. Transfer **30 µL** to a new strip tube.

32 Sample Index PCR

1. Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
2. Add **50 µL** Amp Mix to **30 µL** sample.
3. Add **20 µL** of an individual Dual Index TT Set A to each tube and record assignment.
4. Pipette mix 5X (pipette set to 90 µl) and centrifuge briefly.
5. Incubate in a thermal cycler to incorporate sample indices (run time: approximately 25-40 minutes).

- Use Recommended Cycle Numbers Table (in User Guide) for total number of cycles; recommended number of cycles is based on cDNA input.

6. Store at **4 °C** for up to **72:00:00** or proceed to the next step.

33 Post Sample Index PCR Double Sided Size Selection -- SPRIselect

3d 0h 14m 30s

1. Add **60 µL** SPRIselect reagent (0.6X) to each sample and pipette mix 15X (pipette set to 150 µl).
2. Incubate for **00:05:00** at **Room temperature** .
3. Place on magnet until solution clears. DO NOT discard supernatant.
4. Transfer **150 µL** supernatant to a new tube strip tube.
5. Add **20 µL** SPRIselect reagent (0.8X) to each sample and pipette mix 15x (pipette set to 150 µl).
6. Incubate for **00:05:00** at **Room temperature** .
7. Place on magnet and remove supernatant. DO NOT discard any beads.
8. Add **200 µL** freshly prepared 80% ethanol to the pellet. Wait **00:00:30** before removing ethanol.
9. Repeat step 8 for a total of 2 washes.
10. Centrifuge briefly before returning to magnet and removing any residual ethanol.
11. Air dry for **00:02:00** (DO NOT exceed 2 minutes as this will decrease elution efficiency).
12. Remove from the magnet and elute with **35.5 µL** Buffer EB. Pipette mix 15X.
13. Incubate for **00:02:00** at **Room temperature** .
14. Place the tube strip on the magnet until the solution clears.
15. Transfer **35 µL** to a new strip tube.
16. Store at **4 °C** for up to **72:00:00** or at **-20 °C** for long-term storage.

QC Post Library Construction:

- Quantify Library (e.g. using Qubit dsDNA HS Assay)
- Estimate Library size range (e.g. using TapeStation HS D1000 or BioAnalyzer) - expected size range of 300 to 800 bp, average: 475 bp.

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ATAC Library Sequencing

34 (Optional) MiSeq Sequencing - QC for estimation of library quality and number of nuclei captured

1. Paired End, Single Indexing
    a. Read 1: 50 cycles
    b. i7 Index: 8 cycles
    c. i5 Index: 24 cycles
    d. Read 2: 50 cycles
2. Library Loading
    a. 10X recommended Loading concentration: 10 pM
    b. Optional: 1% PhiX
3. Output
    a. 22-25 million reads

35 NovaSeq Sequencing (target - 50,000-100,000 reads per nucleus)

1. Paired End, Single Indexing
    a. Read 1: 50 cycles
    b. i7 Index: 8 cycles
    c. i5 Index: 24 cycles
    d. Read 2: 50 cycles
2. Library Loading
    a. 10X recommended Loading concentration: 300 pM
    b. Optional: 1% PhiX
3. Output
    a. SP: 650–800 million reads
    b. S1: 1.3–1.6 billion reads
    c. S2: 3.3 –4.1 billion reads
    d. S4: 8-10 billion reads

Gene Expression Library Sequencing

36 (Optional) MiSeq Sequencing - QC for estimation of library quality and number of nuclei captured

1. Paired End, Single Indexing
    a. Read 1: 28 cycles
    b. i7 Index: 10 cycles
    c. i5 Index: 10 cycles
    d. Read 2: 91 cycles
2. Library Loading
    a. 10X recommended Loading concentration: 11 pM
    b. Optional: 1% PhiX
3. Output
    a. 22-25 million reads

37 NovaSeq Sequencing (target - 25,000-50,000 reads per nucleus)

1. Paired End, Single Indexing
    a. Read 1: 28 cycles
    b. i7 Index: 10 cycles
    c. i5 Index: 10 cycles
    d. Read 2: 91 cycles
2. Library Loading
    a. 10X recommended Loading concentration: 300 pM
    b. Optional: 1% PhiX
3. Output

Citation: Kimberly Conklin, Bo Zhang, Amanda Knoten, Dinh Diep, Blue Lake, Sanjay Jain, Kun Zhang 10X Genomics Single-Nucleus Multiome (RNA + ATAC) Assay for Profiling Adult Human Tissues https://dx.doi.org/10.17504/protocols.io.5qpvoby69l4o/v2

a. SP: 650–800 million reads
b. S1: 1.3–1.6 billion reads
c. S2: 3.3 –4.1 billion reads
d. S4: 8-10 billion reads

Cell Ranger Arc Mapping and Analysis Pipeline

38 a. Generate Sample Sheet

Use sample sheet generator provided by 10X Genomics to generate a "SampleSheet.csv":Sample Sheet Generator

b. Generate fastq files

Use **cellranger-arc mkfastq** command.

c. Generate the libraries CSV file which lists all of the fastqs to be analyzed together. There should be a header, "fastqs,sample,library_type", followed by one row per set of fastqs. The first comma separated value is the path to the directory containing the fastq files, the second value is the sample name used for each set of fastqs, the last value specifies whether the fastq files are for "Gene Expression" or "Chromatin Accessibility" analysis.

Example of a CSV file:

fastqs,sample,library_type
/home/jdoe/runs/HNGEXSQXXX/outs/fastq_path,example,Gene Expression
/home/jdoe/runs/HNATACSQXX/outs/fastq_path,example,Chromatin Accessibility

d. Run **cellranger-arc count** for each sample.

Cellranger-arc counts intronic reads by default.

Citation: Kimberly Conklin, Bo Zhang, Amanda Knoten, Dinh Diep, Blue Lake, Sanjay Jain, Kun Zhang 10X Genomics Single-Nucleus Multiome (RNA + ATAC) Assay for Profiling Adult Human Tissues https://dx.doi.org/10.17504/protocols.io.5qpvoby69l4o/v2