Noga Korenfeld , Nicolaj I. Toft , Trine V. Dam , Meital Charni-Natan , Lars Grøntved , Ido Goldstein

Noga Korenfeld , Nicolaj I. Toft , Trine V. Dam , Meital Charni-Natan , Lars Grøntved , Ido Goldstein

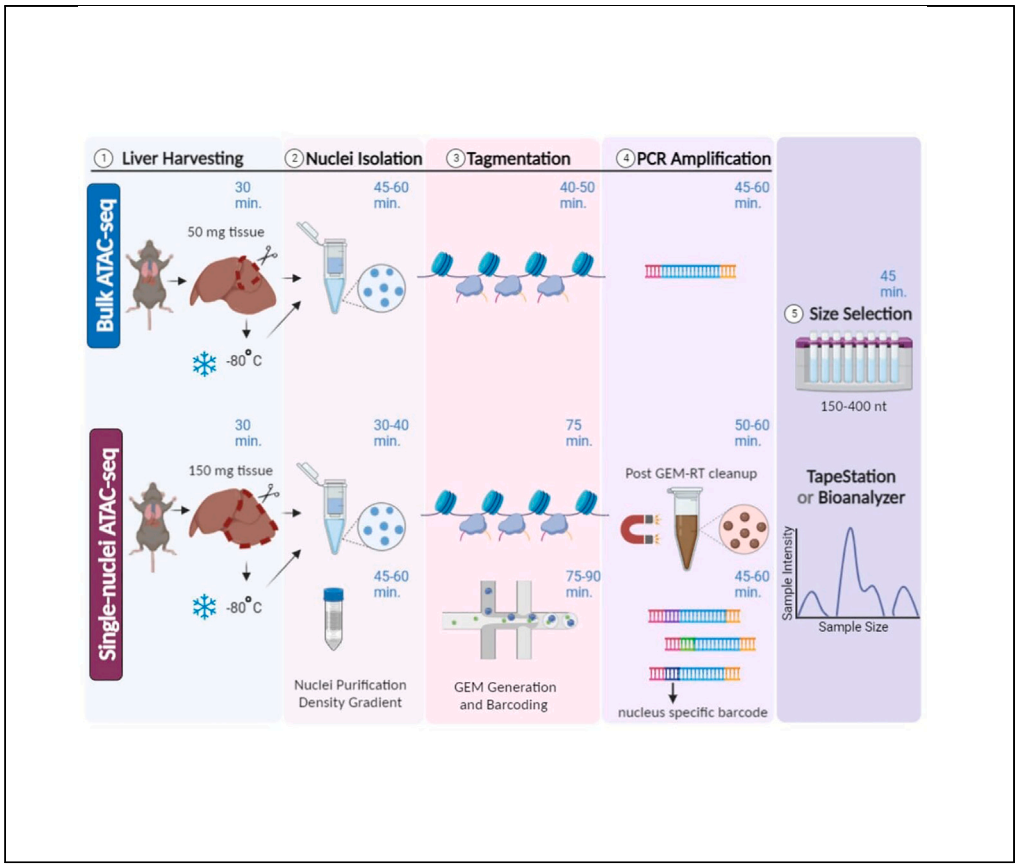
IDRC GRANT / SUBVENTION DU CRDI : - TRANSCRIPTION FACTOR INTERPLAY AS A DRIVING FORCE IN THE HEPATIC RESPONSE TO FASTING AND ITS DYSREGULATION IN METABOLIC DISEASE

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol for bulk and single-nuclei chromatin accessibility quantification in mouse liver tissue



The accessibility of different chromatin regions to transcription factors and other DNA-binding proteins is a critical determinant of cell function. Here, we detail a modified assay for transposase-accessible chromatin sequencing (ATAC-seq) protocol which measures chromatin accessibility genome wide. We describe nuclei isolation, tagmentation, PCR amplification, and pre- and post-sequencing quality control. Our protocol is optimized for the liver, a tissue where nuclei isolation requires distinct steps. We provide two detailed vignettes: one for bulk ATAC-seq and another for single-nuclei ATAC-seq.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Noga Korenfeld, Nicolaj I. Toft, Trine V. Dam, Meital Charni-Natan, Lars Grøntved, Ido Goldstein

larsgr@bmb.sdu.dk (L.G.)
ido.goldstein@mail.huji.ac.il (I.G.)

Highlights

ATAC-seq is a powerful technique to quantify accessible chromatin genome wide

We designed a protocol to address the special requirements of ATAC-seq for liver tissue

We provide two vignettes: one for bulk ATAC-seq and another for single-nuclei ATAC-seq

Special emphasis is put on isolation of high-quality nuclei preparation

Korenfeld et al., STAR Protocols 4, 102462 September 15, 2023
https://doi.org/10.1016/j.xpro.2023.102462

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol for bulk and single-nuclei chromatin accessibility quantification in mouse liver tissue

Noga Korenfeld,[1,3,4] Nicolaj I. Toft,[2,3,4] Trine V. Dam,[2] Meital Charni-Natan,[1] Lars Grøntved,[2,*] and Ido Goldstein[1,5,*]

[1]Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, POB 12, Rehovot 7610001, Israel

[2]Department of Biochemistry and Molecular Biology, University of Southern Denmark, 5230 Odense M, Denmark

[3]These authors contributed equally

[4]Technical contact: noga.korenfeld@mail.huji.ac.il, nicolajit@bmb.sdu.dk

[5]Lead contact

*Correspondence: larsgr@bmb.sdu.dk (L.G.), ido.goldstein@mail.huji.ac.il (I.G.)
https://doi.org/10.1016/j.xpro.2023.102462

SUMMARY

The accessibility of different chromatin regions to transcription factors and other DNA-binding proteins is a critical determinant of cell function. Here, we detail a modified assay for transposase-accessible chromatin sequencing (ATAC-seq) protocol which measures chromatin accessibility genome wide. We describe nuclei isolation, tagmentation, PCR amplification, and pre- and post-sequencing quality control. Our protocol is optimized for the liver, a tissue where nuclei isolation requires distinct steps. We provide two detailed vignettes: one for bulk ATAC-seq and another for single-nuclei ATAC-seq.

BEFORE YOU BEGIN

The protocol below describes the specific steps for ATAC-seq from mouse liver tissue. While this protocol was greatly inspired by previous protocols,[1–3] some aspects of the protocol significantly diverge from earlier bulk and single cell/nuclei ATAC-seq protocols,[1–5] including nuclei preparation and ATAC library size selection. ATAC-seq has gained popularity due to its high reproducibility and the reduced need for a specialized set of skills by the researcher. Indeed, most ATAC-seq steps are not laborious and the protocol is usually smooth-running. One exception is the nuclei isolation step which suffers from vast variability between different starting materials and thus requires specific protocols to obtain high quality nuclei for the downstream tagmentation of chromatin-embedded DNA. This is at odds with the requirement for an extremely 'clean' nuclei population for ATAC-seq. In fact, a clean nuclei prep is indispensable to a successful ATAC-seq protocol.[2] We found that liver tissue requires unique steps in order to isolate high-quality nuclei. This protocol focuses on the specific steps needed for ATAC-seq from liver tissue with a special emphasis on nuclei isolation which is altered from existing protocols. Moreover, we have optimized the ATAC library size selection to obtain a high frequency of tagmented DNA fragments from cis-regulatory regions for downstream sequencing. This allows identification of accessible cis-regulatory regions at lower sequencing depth. Although we have only tested the protocol on liver tissue, most steps should be applicable to other tissues and species. If other tissues are used, we predict that the nuclei preparation will require some optimization.

The sequenced chromatin accessibility data from bulk ATAC-seq is averaged from all tagmented DNA originating from thousands of cells. Therefore, in bulk ATAC-seq one cannot infer population variability, cell type-specific chromatin attributes etc. This is specifically relevant for analysis of

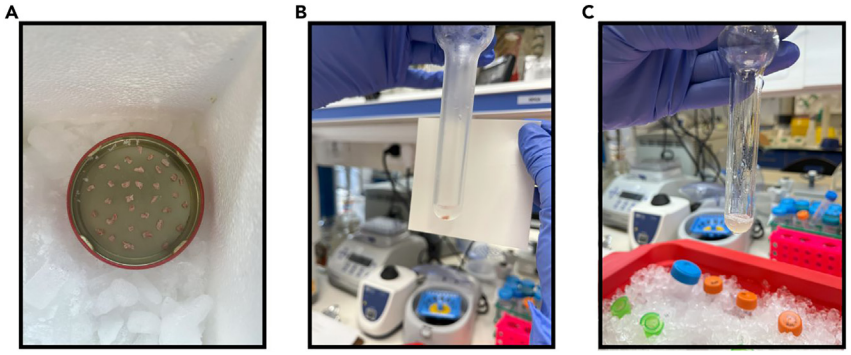


Figure 1. Liver cutting and homogenization

(A–C) Liver pieces are cut and placed on top of a metal plate which is positioned on dry ice. Complete freezing is obtained within seconds (A). Liver pieces are added to ice-cold HB in a Dounce homogenizer (B). The liver sample is homogenized. After homogenization is complete, the liquid appears as an opaque homogenate (C).

tissues where cell populations can change dramatically in response to the physiological or pathophysiological setting. For example, bulk ATAC-seq analysis of inflamed tissue such as in steatohepatitis compared to healthy controls will identify a substantial proportion of differential accessible regions in chromatin that reflects increased abundance of immune cells. Consequently, it will be impossible to discriminate between induced inflammatory response in hepatocytes and increased infiltration of immune cells in the tissue. However, for short term treatments including fasting/fed transitions and circadian experiments, where most abundant cell populations remain largely unchanged, bulk ATAC-seq collects information from many more nuclei at higher sequencing depth with lower costs, and is easier and faster than snATAC-seq. Therefore, the choice between bulk ATAC-seq and snATAC-seq depends on several considerations including experimental design, the biological question and cost.

Tissue preparation

Timing: 30 min

ATAC-seq can be performed on frozen liver tissue. Therefore, tissues can be harvested several days and even months prior to commencement of the ATAC-seq protocol.

1. Excise mouse liver and place it on a Petri dish. Use a scalpel to cut the liver into small pieces of about 50 mg each. Move to step 2 as soon as possible and no longer than 3 min.
2. Freeze liver pieces on dry ice or flash-freeze in liquid nitrogen. Handle liquid nitrogen only in a well-ventilated room. Wear cold insulating gloves, a face shield, and eye protection.

Note: Freezing on dry ice can be done by placing the liver pieces on a sterilized metal plate which is positioned on top of a surface of dry ice. This way, the liver pieces freeze after a few seconds Figure 1A). Be careful not to freeze liver pieces when they are close to each other, this will make it harder to detach them later. Alternatively, freeze the whole liver in liquid nitrogen and use a tissue pulverizer (e.g., steel Bessman Tissue Pulverizer) to fragment tissue to smaller pieces.

Pause point: liver pieces can be stored in −80°C for long term storage. We have successfully used frozen liver stored in −80°C for 6 months.

Institutional permissions

All animal procedures were compatible with the standards for the care and use of laboratory animals. The research has been approved by the Hebrew University of Jerusalem Institutional Animal Care

and Use Committee (IACUC) and by the Danish Animal Inspectorate. Before beginning any animal experiments researchers are required to acquire permissions from the relevant institutions.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Trypan blue solution	Biological Industries	03-102-1B
Tagmentase (Tn5 transposase)	Illumina	20034197
Tagmentase (Tn5 transposase) buffer	Illumina	20034197
Nuclease-free phosphate-buffered saline (PBS)	Sartorius	02-023-1A
Nuclease-free water	Hylabs	BP731/500D
Digitonin	Sigma	D141-100MG
Tween-20	Avantor	X251-07
MinElute PCR purification kit	Qiagen	20-28006
Q5 High-Fidelity DNA Polymerase	New England Biolabs	M0491L
Q5 Reaction Buffer	New England Biolabs	B9027S
PCR dNTP's Mix	Larova	VAR-02
Agencourt AMPure XP beads	Beckman Coulter	BC-A63881
Ethanol, absolute	Various sources	N/A
Qubit™ 1× dsDNA High Sensitivity (HS) and Broad Range (BR) Assay Kits	Thermo Scientific	Q33231
Chromium Next GEM Single Cell ATAC Library Kit v1.1	10× Genomics	1000163
Chromium Next GEM Single Cell ATAC Gel Bead Kit v1.1	10× Genomics	1000159
Dynabeads™ MyOne™ SILANE	10× Genomics	2000048
Chromium Next GEM Chip H Single Cell Kit v1.1	10× Genomics	1000162
OptiPrep™ Density Gradient Medium,	Sigma	D1556
Glycerin (glycerol), 50% (v/v) Aqueous Solution	RICCA Chemical Company	3290-32
SPRIselect beads	Beckman Coulter	B23317
Experimental models: Organisms/strains		
Mouse strain: C57BL/6JOlaHsd Mouse sex: male or female Mouse age: we recommend using 8–12 week old mice, unless experimental design requires otherwise		
Oligonucleotides		
Primers for library preparation, see Table 1		
Single Index Kit N Set A	10× Genomics	1000212
Other		
Dounce homogenizer and pestles	Capitol Scientific	357542
40 µm filter	PluriSelect	43-50040-51
Magnetic rack	Invitrogen	12321D
Qubit fluorometer	Thermo Scientific	Q33240
ProFlex Thermocycler to conduct PCR	Thermo Scientific	N/A
Microcentrifuge	Various sources	N/A
Swinging bucket centrifuge	Various sources	N/A
Thermomixer	Eppendorf	5382000031
Pipette Tips RT UNV 1000µL FL 768A/8	Mettler Toledo	30389166
Pipette Tips RT UNV 200µL FL 960A/10	Mettler Toledo	30389187
Pipette Tips RT UNV 100µL FL 960A/10	Mettler Toledo	30389171
Pipette Tips RT UNV 20µL FL 960A/10	Mettler Toledo	30389190
Pipette Tips RT UNV 10µL FL 960A/10	Mettler Toledo	30389173
Pipet-Lite Multi Pipette L8-50XLS+	Rainin	17013804
Pipet-Lite Multi Pipette L8-200XLS+	Rainin	17013805
Pipet-Lite LTS Pipette L-10XLS+	Rainin	17014388
Pipet-Lite LTS Pipette L-20XLS+	Rainin	17014392
Pipet-Lite LTS Pipette L-100XLS+	Rainin	17014384

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Pipet-Lite LTS Pipette L-200XLS+	Rainin	17014391
Pipet-Lite LTS Pipette L-1000XLS+	Rainin	17014382
LoBind tubes 1.5 mL	Eppendorf	022431021
10× chromium controller	10× Genomics	1000204
10× vortex adapter	10× Genomics	120251
Chromium Next GEM Secondary Holder	10× Genomics	1000195
10× magnetic separator	10× Genomics	120250
Open-top polyallomer centrifuge tubes, 12 mL	Seton	5030
40 µm pipette tip filter	SP Bel-Art	136800040
Sorvall TH-641 swinging bucket rotor	Thermo Scientific	54295
Sorvall WX 80+ Ultracentrifuge	Thermo Scientific	75000080

MATERIALS AND EQUIPMENT

Hypotonic Buffer (HB)

Reagent	Final concentration	Amount
Sucrose	250 mM	1.09 gr
Tric HCl pH 7.8 1 M	10 mM	100 µL
Igepal 10%*	0.1%	100 µL
$MgCl_2$ 1 M	3 mM	30 µL
KCl 3M	10 mM	33.3 µL
Dithiothreitol (DTT)** 1 M	0.2 mM	2 µL
Spermidine** 0.5 M	0.5 mM	10 µL
Protease Inhibitor Cocktail**	1:100	50 µL
Nuclease-free water	N/A	9.675 mL
Total		**10 mL**

*Some samples or experimental setting may be sensitive to detergents such as Igepal. It is optional to use 2% bovine serum albumin (BSA) instead of Igepal.
**Add fresh on the day of experiment.
Store at 4°C for up to 3 months.

Nuclei Preparation Buffer (NPB)*

Reagent	Final concentration	Amount
Tric HCl pH 7.5 1 M	10 mM	500 µL
$MgCl_2$ 1 M	3 mM	150 µL
NaCl 5 M	10 mM	100 µL
Nuclease-free water	N/A	49.250 mL
Total		**50 mL**

*In some steps, NPB is supplemented with 0.1% Tween-20, see main text.
Store at 4°C for up to 6 months.

Tagmentation reaction mix (volume shown is for one reaction)

Reagent	Final concentration	Amount
nuclease-free PBSx1	N/A	16.5 µL
Tween-20 10%	0.1%	0.5 µL
Digitonin 1%*	0.01%	0.5 µL
Nuclease-free water	N/A	5 µL
Total		**22.5 µL**

*Briefly pre-heat digitonin to 65°C to solubilize it.
Make fresh at the day of experiment.

Table 1. Indexing primers for tagmentation[a]
Ad1_noMX: AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1_TAAGGCGA CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2_CGTACTAG CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3_AGGCAGAA CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4_TCCTGAGC CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5_GGACTCCT CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6_TAGGCATG CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7_CTCTCTAC CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8_CAGAGAGG CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9_GCTACGCT CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10_CGAGGCTG CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11_AAGAGGCA CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12_GTAGAGGA CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13_GTCGTGAT CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14_ACCACTGT CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15_TGGATCTG CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16_CCGTTTGT CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.17_TGCTGGGT CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
Ad2.18_GAGGGGTT CAAGCAGAAGACGGCATACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
Ad2.19_AGGTTGGG CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT
Ad2.20_GTGTGGTG CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGT
Ad2.21_TGGGTTTC CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
Ad2.22_TGGTCACA CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23_TTGACCCT CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT
Ad2.24_CCACTCCTCAAGCAGAAGACGGCATACGAGATAGGAGTGGGTCTCGTGGGCTCGGAGATGT

[a] The primers are not part of the PCR kit nor the Tagmentase kit. They should be purchased separately.

STEP-BY-STEP METHOD DETAILS

Bulk ATAC-seq

In bulk ATAC-seq nuclei are isolated from liver tissue followed by tagmentation, PCR amplification, DNA library size selection, and post-library quality control. The data represents an average from all tagmented DNA originating from all cells in a population. In liver this will mostly be hepatocytes.

Liver nuclei preparation

Timing: 45–60 min

Here, the nuclei are isolated from mouse liver tissue. A clean nuclei population, deprived of cellular debris and free-floating genomic DNA, is *essential* for high quality ATAC-seq results. The work can be done in an open bench top.

> *Note:* Both bulk and snATAC-seq have nuclei isolation steps. While some steps are similar, there are considerable differences between the two protocols. This is due to the need for an extremely clean nuclei prep in snATAC-seq which necessitates a density gradient or similar nuclei cleanup methods. Therefore, it is advised to use the appropriate nuclei isolation steps depending on the type of protocol chosen (bulk or single-nuclei).

1. Cool a 7 mL Dounce homogenizer on ice.
2. Prepare HB, NPB and NPB + 0.1% tween-20. Keep on ice.
3. Add 500 µL HB to the Dounce homogenizer, then add 50 mg of frozen liver tissue directly to the Dounce homogenizer (Figure 1B).

4. Homogenize the tissue by 15 strokes with the loose pestle ('A'), followed by 15 strokes with the tight pestle ('B'). Avoid bubbles during homogenization. After homogenization, the liquid appears as an opaque homogenate (Figure 1C).

⚠ CRITICAL: Number/roughness of douncing greatly affects the number, quality and intactness of nuclei released. Different Dounce homogenizers provide variable results. Optimize number of strokes for your specific Dounce homogenizer and if possible, use the same Dounce homogenizer across different experiments.

5. Filter homogenate through a 40 μm filter to a 1.5 mL tube on ice (no pre-wetting of the filter with buffers is needed). A strainer of 40 μm allows passage of larger multiploidy nuclei from subsets of hepatocytes.
6. Wash the Dounce homogenizer with another 500 μL of HB and pass the collected material through the same 40 μm filter (as in step 5) to the 1.5 mL tube containing the sample.
7. Spin 500 g / 30 s / 4°C, save flow-through and discard filter.

Note: This short centrifugation step helps the dense solution to go through the filter quickly rather than waiting for it to flow through the filter by gravity alone.

8. Spin 500 g / 10 min / 4°C, aspirate supernatant. The pellet contains your nuclei.

Note: The pellet should appear white or transparent.

9. Gently wash nuclei in 1 mL NPB + 0.1% tween-20 using a P1000, pipetting 10 times. Spin 500 g / 10 min / 4°C, discard supernatant gently using a P1000.
10. Gently resuspend nuclei in 100 μL NPB and keep on ice.
11. Take a 5–10 μL from the sample and dilute 1:1 with trypan blue. Count nuclei using a hemocytometer. Nuclei should appear round and intact (Figure 2).
12. Transfer 5×10^4 nuclei to a new 1.5 mL tube.

Note: Bring the total volume of each sample to at least 20 μL with NPB.

13. Spin 500 g / 10 min / 4°C, aspirate supernatant. Keep on ice. It is preferable to continue immediately to the next steps. However, it is possible to freeze nuclei in −80°C for up to a month.

Tagmentation and purification

Timing: 40–50 min

Here, the tagmentase (Tn5 transposase loaded with sequencing adapters) enzyme cuts the DNA preferentially at accessible regions and inserts sequencing adapters at the flanks of the cut fragments.

14. Resuspend nuclei pellet in 22.5 μL of tagmentation reaction mix (detailed in materials and equipment).
15. To each sample add:
    a. 25 μL tagmentase buffer.
    b. 2.5 μL tagmentase enzyme.
16. Mix gently by pipetting 20 times.

Note: mix well to introduce tagmentase to all nuclei.

17. Incubate the tagmentation reaction with shaking in the Thermomixer at 37°C / 30 min / 1000 rpm.

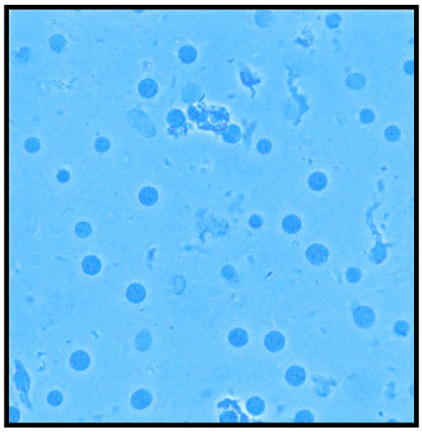


Figure 2. Nuclei isolation evaluation
Following the nuclei isolation steps in bulk ATAC-seq protocol, nuclei are stained with trypan blue and assessed for intact-ness. Some debris are apparent while most of material consists of round, intact nuclei.

18. Immediately following tagmentation, short spin samples and purify DNA using a MinElute PCR purification kit following manufacturer's instructions. Elute DNA in 21 µL Nuclease-free water.

Note: It is important to purify your samples immediately after incubation in order to stop the tagmentation reaction and avoid over-tagmentation.

Pause point: store samples at −20°C for up to a week.

PCR amplification

Timing: 45–60 min

Here, the tagmented DNA fragments are amplified, with each sample barcoded with a unique index. At the end of the protocol, those indices will be used to identify which sequencing reads stem from which sample as samples are typically pooled during sequencing.

19. Transfer 10 µL of the purified tagmented DNA to a 0.2 mL PCR tube on ice.

Note: 11 µL of the purified tagmented DNA are left unused. The unused DNA can be frozen until the end of the experiment. It could be used if errors are made in the rest of the protocol (e.g., wrong indexing).

20. Add 1 µL of individual indexing primers (Ad2 type primers, see Table 1) to each 10 µL sample in PCR tubes.

⚠ CRITICAL: To allow pooling during sequencing, each sample should get a unique index. Record which index was allocated to which sample.

21. Prepare with excess and add 13 µL of PCR reaction master mix to each indexed sample:

PCR reaction master mix (volume shown is for one reaction)

Reagent	Amount
Ad1_noMX primer 25 µM*	1 µL
Polymerase enzyme Q5	0.25 µL
Q5 Reaction buffer	5 µL

(*Continued on next page*)

Continued	
Reagent	Amount
dNTPs	0.5 μL
Nuclease-free water	6.25 μL
Total	13 μL

*This primer is used in all samples. Only the indexing primers alter between samples (see Table 1).

22. Run PCR as described in the table below:

PCR cycling conditions

Steps	Temperature	Time	Cycles
Initial extension	72°C	5 min	1
Initial denaturation	98°C	30 s	
Denaturation	98°C	15 s	12
Annealing	60°C	30 s	
Extension	72°C	1 min	
Hold	4°C	Hold	

23. Purify the PCR-amplified tagmented DNA using a MinElute PCR purification kit following manufacturer's instructions. Elute DNA in 21 μL nuclease-free water.

Pause point: store samples at −20°C for up to 1 week.

Size selection

Timing: 45 min

Here, the purified DNA fragments of a desired size range are isolated, using designated DNA cleanup beads (most commonly used are magnetic AMPure XP beads and SPRIselect beads). At the end of this step, DNA fragments, including index and sequencing adapters, ranging from 150nt to 400nt are isolated and are ready for quality control and sequencing. This primarily corresponds to nucleosome depleted DNA and mono-nucleosomal DNA.

Note: size selection is optional and enriches the tagmented DNA library for DNA fragments originating from non-nucleosomal DNA. If the aim is to analyze nucleosomal DNA, size selection should be modified or avoided.

Note: Before starting size selection steps, prepare 80% ethanol (400 μL per sample).

24. Add 29 μL of nuclease-free water to the 21 μL purified DNA from the previous step, to reach a total volume of 50 μL.
25. Mix AMPure XP beads (or SPRIselect beads) thoroughly by vortexing for a few seconds and add 32.5 μL beads to each 50 μL sample. Mix thoroughly by gently pipetting 10 times.
26. Incubate at 22°C–25°C for 5 min.
27. Place the reaction tubes on the magnetic rack for 5 min.
28. Move the supernatant carefully to a new tube and discard the beads.
29. Mix AMPure XP beads (or SPRIselect beads) thoroughly by vortexing for a few seconds and add 88 μL beads to the supernatant. Mix thoroughly by gently pipetting 10 times.
30. Incubate bead/sample mixture at 22°C–25°C for 5 min.
31. Place the reaction tube on the magnetic rack for 5 min.

32. Remove the supernatant carefully and discard.
33. Keep the tubes in the magnetic rack and add 200 μL of freshly prepared 80% ethanol to the samples.
34. Incubate at 22°C–25°C (still on the magnetic rack) for 30 s and discard the supernatant using a pipettor.
35. Repeat steps 33 and 34 once more (i.e., wash again with 80% ethanol).
36. Keep the tubes in the magnetic rack, open the tube lid and air-dry the beads for 3 min.
37. Take out the tubes from magnetic rack and add 20 μL of nuclease-free water to the beads to elute. Mix by gently pipetting 10× times.
38. Short spin the tube and place in the magnetic rack. Let the tube rest for 5 min.
39. Transfer supernatant to a new 0.2 mL PCR tube without disturbing/collecting any of the beads.
40. Check DNA concentration with 1 μL of sample on a Qubit fluorometer.

Note: Optimally, sample concentration should be above 1 ng / μL.

Pause point: store samples at −20°C for up to 1 month.

The samples are now ready for post-library quality control (see 'expected outcomes').

Single-nuclei ATAC-seq

In snATAC-seq nuclei are isolated from liver tissue followed by tagmentation, generation of gel beads-in-emulsion (GEM), barcoding, cleanup, indexing, PCR amplification and size selection. snATAC-seq provides genome-wide chromatin accessibility data from each cell. Thus, giving insights into heterogeneity within cell populations as well as cell type-specific data. However, the snATAC-seq protocol is longer and the cost is much higher compared to bulk ATAC-seq. Using the current 10× Genomics platform, one snATAC-seq library reaction using 10,000 nuclei as input, costs about twenty times more than a bulk ATAC-seq library for 50,000 nuclei. For sequencing costs alone snATAC-seq is about eight times as expensive as bulk ATAC-seq, given that at least 30 million reads are required for bulk ATAC-seq, whereas 250 million reads are required for snATAC-seq (if 10,000 nuclei are sampled). Therefore, deciding between bulk ATAC-seq or snATAC-seq depends on several considerations including experimental design, the biological question and cost.

Note: The snATAC-seq library preparation was performed using the Chromium 10× controller and the single cell ATAC v1.1 kit. The protocol describing GEM generation, Chromium operation, post GEM processing and library construction is identical to the standard protocol for the ATAC v1.1 kit and we refer the user to the online detailed protocol. We have optimized nuclei purification, made slight modifications to the tagmentation step and optimized the DNA library size selection providing a larger fraction of sequenced reads from nucleosome depleted cis-regulatory regions.

Liver nuclei preparation

Timing: 30–40 min

Here, the nuclei are isolated from mouse liver tissue and filtered for subsequent purification using gradient centrifugation.

Note: Both bulk and snATAC-seq have nuclei isolation steps. While some steps are similar, there are considerable differences between the two protocols. This is due to the need for a very clean nuclei prep in snATAC-seq which necessitates a density gradient. Therefore, it is recommended to use the appropriate nuclei isolation steps depending on the type of protocol chosen (bulk or single-nuclei).

Note: Prepare everything that can be prepared in advance so that nuclei are processed as quickly as possible (e.g., prepare the different dilutions of gradient buffers beforehand).

41. Cool a 7 mL Dounce homogenizer and pestles A and B on ice.
42. Prepare HB. Keep on ice.
43. Add 2 mL HB to the Dounce homogenizer, then add 150 mg of liver tissue directly to the Dounce homogenizer and wait a few seconds until tissue is thawed.

Note: In snATAC-seq much more starting material is needed compared with bulk ATAC-seq (150 mg vs 50 mg, respectively). This is due to the extra density gradient purification of the nuclei which requires higher amount of starting material.

44. Homogenize the tissue by 15 strokes with the loose pestle A, followed by 15 strokes with the tight pestle B.

⚠ CRITICAL: Number/roughness of douncing greatly affects the number, quality and intactness of nuclei released. Different Dounce homogenizers provide variable results. Optimize number of strokes for your specific Dounce homogenizer and if possible, use the same Dounce homogenizer across different experiments. Insufficient Dounce homogenization will result in low nuclei yield and compromised nuclei quality. This will be evident after purification using density gradient. If nuclei quality is compromised this will result in small number of nuclei passing quality control after sequencing.

45. Filter suspension through a 40 μm strainer to a 50 mL tube. A strainer of 40 μm allows passage of larger multiploidy nuclei from subsets of hepatocytes.
46. Transfer homogenate to a 15 mL tube and spin 400 g / 5 min / 4°C.

Nuclei purification by a density gradient

Timing: 45–60 min

Here, a density gradient is used to purify nuclei, getting rid of cellular debris and free-floating genomic DNA from the lysis which can confound the downstream snATAC-seq data. This results in higher purity compared to only filtering. At the end of this step, high quality nuclei are isolated and ready for tagmentation.

47. Gently resuspend nuclei pellet in 2 mL cold HB by pipetting 10 times.

Optional: When setting up the protocol we would recommend counting the nuclei here as a quality control (see step 55).

Note: Nuclei are now ready for density gradient purification. Keep nuclei on ice while density gradient is prepared. We usually see between 15–30 million nuclei at this step, depending on the amount of liver used.

48. Prepare 1× Nuclei buffer from the snATAC kit (will be used in step 57 below), and store on ice (the amount below is per one sample):
    a. 50 μL 20× Nuclei buffer.
    b. 950 μL nuclease-free water.
49. Prepare 3 different concentrations of OptiPrep suspension (50%, 40% and 30%) by mixing the OptiPrep stock with nuclease-free water according to the scheme below (calculated for 2 samples + surplus). Keep all tubes on ice.

Note: prepare appropriate number of tubes to balance the centrifuge.

	OptiPrep stock (60%)	Nuclease-free water
50% suspension	3.75 mL	0.75 mL
40% suspension	5 mL	2.5 mL
30% suspension	3.75 mL	3.75 mL

50. For each nuclei sample: mix 2 mL nuclei suspension with 2 mL of prepared 50% OptiPrep suspension (keep on ice). This is your 25% OptiPrep + nuclei suspension for later usage in the gradient top layer (step 51.c).
51. For each nuclei sample: build a 3-layer OptiPrep density gradient in a 12 mL open-top centrifuge tube (keep on ice) (See Methods video S1).
    a. Add 3.5 mL 40% OptiPrep suspension to the bottom of the tube.
    b. Carefully add 3.5 mL 30% OptiPrep suspension on top of the bottom layer.
    c. Carefully add 4 mL 25% OptiPrep + nuclei suspension on top of the middle layer.

⚠ CRITICAL: When preparing the gradient, release suspensions on the side of the tube. This allows the liquid to land gently on the liquid surface (Figure 3A, Methods video S1).

52. Centrifuge the gradient tubes in an ultracentrifuge using swinging-buckets at 10,000 g / 24 min / 4°C (no break). After centrifugation, the purified nuclei are located between the bottom and middle layer and appears as a cloudy layer (Figures 3B and 3C).
53. Extract the purified nuclei band:
    a. Carefully, remove the liquid from the top of the gradient tube until the liquid surface is 1–2 cm from the nuclei band.
    b. Use a P1000 pipette to carefully extract the cloudy nuclei band into a new 15 mL tube (~1–1.5 mL is usually extracted). (See Methods video S2).
54. Wash the nuclei:
    a. Add 4 mL of HB to the purified nuclei and mix by pipetting 10 times (total volume in tube ~5–6 mL).
    b. Spin down nuclei at 500 g / 5 min / 4°C. Discard supernatant.
    c. Resuspend the nuclei pellet in 1 mL HB. Transfer to a 1.5 mL tube. This is your purified nuclei suspension ready for next steps. We have not done any experiments with cryopreserved nuclei and recommend to proceed directly to tagmentation.

Note: The optimal resuspension volume depends on nuclei quantity. If you observe very low quantity either by counting the nuclei in step 47 or observe a less turbid band than shown in Figures 3B and 3C, lowering the resuspension volume in step 54c is recommended. If you count 7 million nuclei in step 47, resuspension volume can be halved.

Tagmentation

Timing: 75 min

Here, nuclei aliquots for tagmentation are prepared and tagmentation is performed. At the end of this step, DNA within nuclei is tagmented.

Note: Work in biological cabinet hood and use gloves, make sure to use a lab coat and avoid clothes that can shed fibres. Use specialized pipettes and pipette tips (see key resources table) at all steps leading up to running the Chromium controller. Other pipette tips might shed fibres and clog the chip.

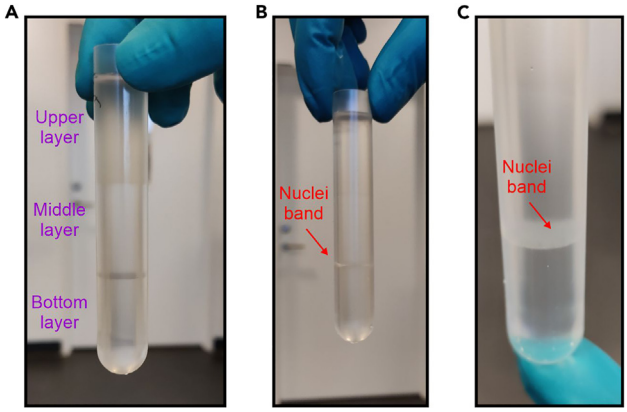


Figure 3. Density gradient-based nuclei purification

(A–C) In snATAC-seq, nuclei are purified by a 3-layer density gradient (A). After centrifugation, the nuclei are gathered between the bottom and middle layers (B and C).

55. Take 10 μL of nuclei sample and dilute 1:1 with trypan blue. Count nuclei using a hemocytometer. Nuclei should appear round and intact (Figure 4).

Note: At this step we usually observe 3–10 million nuclei after the gradient.

56. Centrifuge nuclei from 54c at 500 g / 5 min / 4°C. Discard supernatant.
57. Resuspend each pellet in 1× Nuclei buffer (from snATAC-seq kit, prepared in step 48) to reach the nuclei concentration outlined below in step 57a and pass through SP Bel-Art 40 μm pipette-tip filter. Collect in a 1.5 mL Eppendorf tube.
    a. The volume is calculated as follows: $V_{NB_{1x}} = \frac{Amount\ of\ nuclei}{7700 \frac{nuclei}{\mu L}}$

       Example calculation of resuspension volume with 5 million nuclei:

$$V_{NB_{1x}} = \frac{5,000,000\ nuclei}{7700\ \frac{nuclei}{\mu L}} = 649.35 \mu L$$

    b. The 7700 nuclei / μL is the upper limit of final concentration recommended by 10× Genomics for recovery of 10,000 nuclei at the end. The upper limit is used because some nuclei are lost during resuspension and filtering.

Note: The last filtration step avoids any clogs in the Chromium Next GEM Chip H.

58. Count nuclei again (see step 55).
59. Aliquot 12,000 nuclei for tagmentation for target recovery of 10,000 nuclei.
60. Add 1× Nuclei Buffer up to 5 μL.

Note: Use 12,000 nuclei for tagmentation reactions in a volume of 5 μL. Exceeding 12,000 nuclei will dramatically increase the number of nuclei doublets during the droplet formation in the Chromium controller.

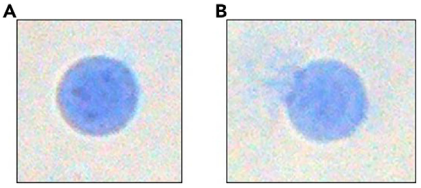


Figure 4. Examples of purified nuclei

(A and B) An intact nucleus (A) and a broken nucleus that is leaking chromatin (B) are shown. Broken nuclei may compromise the quality of the ATAC.

61. Transfer the 5 µL nuclei sample (containing 12,000 nuclei) to a PCR strip, keep on ice.
62. Prepare tagmentation mix, mix by pipetting 10 times.

Tagmentation mix	1 rxn (µL)	4 rxns + 10% (µL)	8 rxns + 10% (µL)
ATAC Buffer (from ATAC v1.1 kit)	7	30.8	61.6
ATAC Enzyme (from ATAC v1.1 kit)	3	13.2	26.4
Total	**10**	**44**	**88**

63. Add 10 µL tagmentation mix to each nuclei sample and mix by pipetting gently 10 times. Do not spin down.
64. Incubate in thermal cycler using the following protocol:

Lid temperature	Reaction volume	Run time
50°C	15 µL	60 min
Step	**Temperature**	**Time**
1	37°C	60:00
2	4°C	Hold

GEM generation and barcoding

Timing: 75–90 min

In this step the 10× barcoded gel beads are emulsified with tagmented single nuclei. Subsequently a PCR is performed to linearly amplify and barcode DNA fragments. The result is a solution consisting of gel beads-in-emulsion (GEMs) with linearly amplified and barcoded DNA fragments, where each emulsion has a unique barcode. To complete this step in the protocol we refer to the 10× protocol supplied with the ATAC v1.1 kit used together with the 10× Chromium controller. It can also be found online.

Pause point: store samples at −20°C up to 1 week.

Post GEM-RT cleanup

Timing: 50–60 min

In this step the emulsions are broken, lipids are removed and the linearly amplified DNA from different emulsions are pooled. This is followed by cleanup of the DNA. The result of this step is a pool of DNA fragments where each pool of fragments derived from a GEM will have a specific barcode. Please use the detailed protocol supplied by the ATAC v1.1 kit.

65. This step results in a 40 µL bead-purified Post Ligation DNA library.

Pause point: keep samples at 4°C for up to 72 h or at −20°C for up to 2 weeks.

Library construction – Sample index PCR

Timing: 45–60 min

In this step the pooled barcoded DNA fragments are indexed with a sample index primer along with linear amplification. The result of this step is DNA fragments with a barcode (corresponding to a specific GEM) and a sample index sequence (common for all DNA fragments from one sample). Please use the detailed protocol supplied by the ATAC v1.1 kit.

Note: We recommend to use half of the library from step 65 for indexing and amplification. This allows one to re-do this step if the library is over- or under-amplified.

Sample index PCR mix	1 rxn (µL)	1/2 volume
Amp Mix	50	25
SI-PCR Primer B	7.5	3.75
Total	57.5	28.75

Lid temperature	Reaction volume	Run time
105°C	50 µL	~30 min
Step	**Temperature**	**Time**
1	98°C	0:45
2	98°C	0:20
3	67°C	0:30
4	72°C	0:20
5	See table below for # of cycles	
6	72°C	1:00
7	4°C	Hold

66. Prepare Sample Index PCR mix, mix thoroughly and centrifuge briefly:

67. For each reaction, add 28.75 µL Sample Index PCR Mix to a fresh PCR tube and add 20 µL bead-purified Post Ligation DNA (step 65).
68. Add 1.25 µL of an individual Chromium i7 Sample Index to each tube and record which sample was assigned which index.

Note: Choose the appropriate sample index sets to make sure that no sample indices overlap in a multiplexed sequencing run.

69. Pipette mix 15 times using a multichannel pipette.
70. Transfer the remaining 20 µL purified Post Ligation sample from step 65 to a 1.5 mL tube. Save in −20°C.

Note: This allows a re-run PCR with fewer or more cycles if the samples are over- or under-amplified.

71. Index the library DNA in a thermal cycler with the following program:

Targeted nuclei recovery	Total cycles
500–2,000	13
2,001–6,000	12
6,001–10,000	11

Size selection

Timing: 45 min

This step uses a size selection setup that removes most of the tagmented fragments from mono-, di-, and tri-nucleosomes. Thus, the library primarily contains tagmented fragments from nucleosome-free regions and thus allows identification of putative cis-regulatory regions at single cell resolution.

72. Vortex the SPRIselect beads until fully resuspended, add 32.5 μL to each sample from PCR reaction and pipette mix 15 times using a multichannel pipette.
73. Incubate the strip at 22°C–25°C for 5 min.
74. Place the tube strip in the Magnetic Separator in the High position until the solution is clear.
75. Transfer supernatant to a new strip and discard the previous strip containing the beads.
76. Add 88 μL SPRIselect to each sample and pipette mix 15 times using a multichannel pipette.
77. Incubate the strip at 22°C–25°C for 5 min.
78. Place the tube strip in the Magnetic Separator in the High position until the solution is clear.
79. Using a pipette, carefully remove and discard supernatant.
80. Add 200 μL 80% ethanol and let it stand for 30 s.
81. Using a pipette, carefully remove and discard the ethanol.
82. Repeat wash steps 80 and 81 for a total of 2 ethanol washes.
83. Centrifuge the strip briefly and return it to the Magnetic Separator, remove and discard the remaining ethanol.
84. Remove tube strip from Magnetic Separator and add 20.5 μL nuclease-free water, mix using a multichannel pipette 15 times.
85. Incubate the strip at 22°C–25°C for 2 min.
86. Place the tube strip in the Magnetic Separator in the High position until the solution is clear.
87. Transfer 20 μL of sample to a new 1.5 mL LoBind Eppendorf tubes.

Pause point: store at −20°C for up to 1 month.

EXPECTED OUTCOMES

Post library quality control

Run 1 μL sample in 29 μL nuclease-free water on a fragment analyzer (e.g., Tapestation or Bioanalyzer). Ideally, most DNA should be around 200nt with a fluorescence unit (FU) significantly higher than the background (Figure 5).

Illumina sequencing

For bulk ATAC-seq: The sequencing should be in paired-end and 30–50 million reads per sample (in ATAC, some sequencing reads map to mitochondrial DNA, see below). Use 38 bp read length (Illumina platform).

For snATAC-seq: The sequencing should be at least 25,000 paired reads (Illumina platform) per nucleus loaded to the 10× chromium.

Post sequencing quality control of ATAC libraries

To evaluate quality of bulk ATAC-seq libraries, the following measures should be considered. First, the size distribution of the sequenced fragments should be evaluated and should reflect the library size measured before sequencing (Figure 5). Fragments less than 150nt largely represent DNA from accessible cis-regulatory regions (Figure 6A). Note that fragment size distribution is highly dependent on the size selection step during library preparation and thus can vary among ATAC-seq libraries. Second, the number of mitochondrial reads should be evaluated. For ATAC-seq protocols without any nuclei purification steps, where permeabilization of cells is the method of choice, a high proportion of reads from mitochondrial (up to 60% of all reads) can be observed.[6] Consequently, the

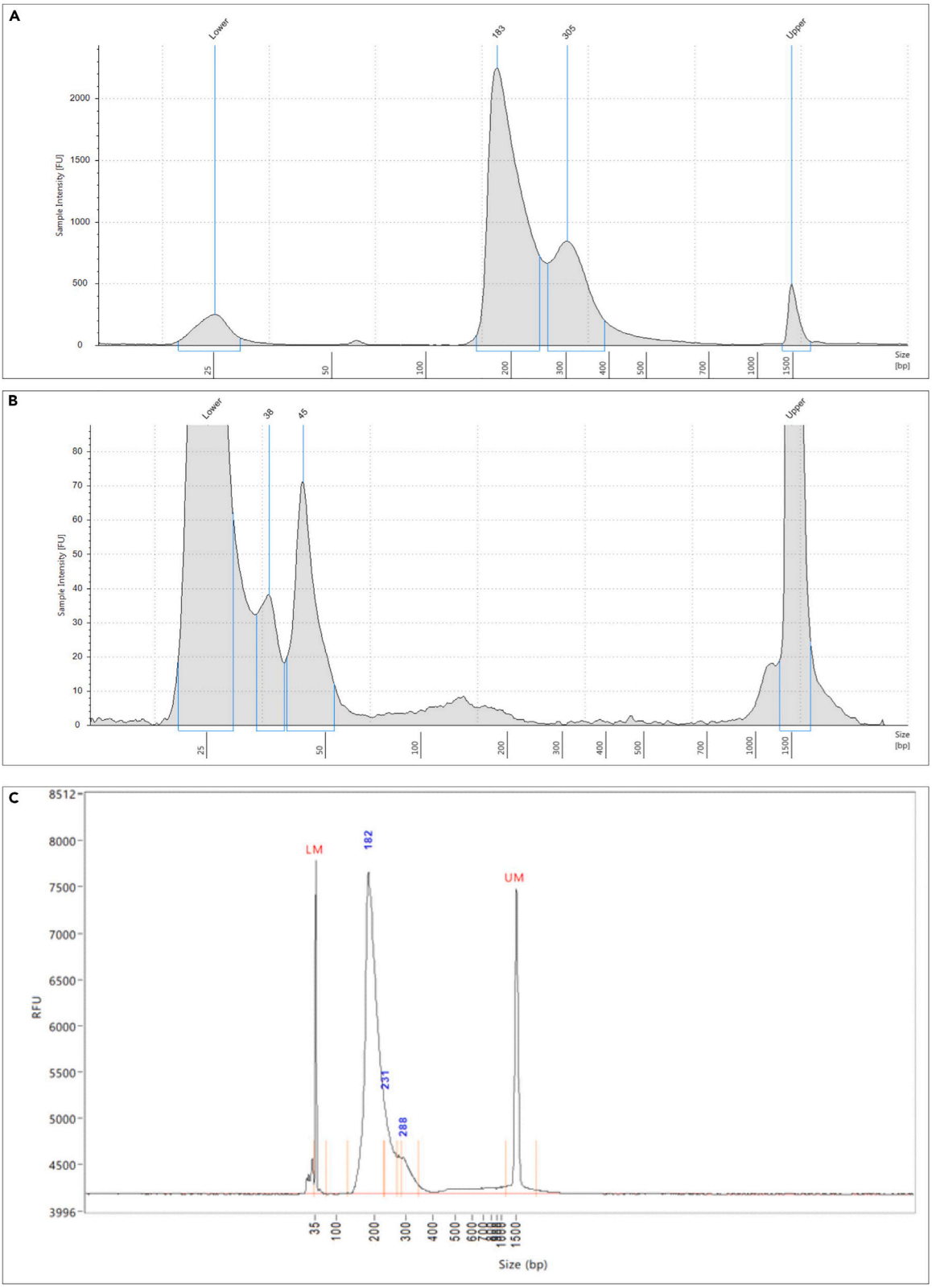

A
Lower
183
305
Upper
Sample Intensity [FU]
2000
1500
1000
500
0
25
50
100
200
300
400
500
700
1000
1500
Size [bp]
B
Lower
38
45
Upper
Sample Intensity [FU]
80
70
60
50
40
30
20
10
0
25
50
100
200
300
400
500
700
1000
1500
Size [bp]
C
8512
8000
7500
7000
6500
6000
5500
5000
4500
3996
RFU
LM
182
UM
231
288
35
100
200
300
400
500
600
1500
Size (bp)

Figure 5. Results of post-library quality control

(A–C) A sample of DNA from a prepared library was run on a fragment analyzer (Tapestation). In an expected result, the majority of DNA species are 150–400nt due to size-selection steps (A). This allows for sub-nucleosomal and mono-nucleosomal DNA to be observed (the mono-nucleosomal DNA + linker DNA + adapter length reaches 300–400nt). Di-nucleosomal DNA sizes and above are not observed due to size selection steps. If DNA sizes are significantly higher or lower than these values, ATAC-seq results might be compromised. This may result from sub-optimal size selection. An unsuccessful library due to low-quality nuclei or inefficient tagmentation will result in very low fluorescence reads in the 150–400nt range (B). Size distribution pattern in snATAC-seq (C) is very similar to bulk ATAC-seq (A).

library must be sequenced to higher depths to detect accessible regions. Libraries generated by the above-described protocol has 4%–16% of mitochondrial reads (Figure 6B). Third, bulk ATAC-seq libraries can be evaluated by number of detected accessible regions (peaks) by software such MACS[7] and later developments of this (MACS2 and 3). The number of peaks detected should be > 80,000 at sequencing depth of >30 million reads (Figure 6C) and the fraction of reads in these peaks should be above 20% for ATAC-seq with high signal to noise ratio (Figure 6D). For in depth information on post-sequencing quality control, we refer to the readers to an excellent summary.[2]

Single nuclei ATAC-seq quality is evaluated with other sets of analysis. Initially the snATAC-seq data can be analyzed by the 10× Genomics Cell Ranger software or by other non-commercial snATAC-seq software such as ArchR.[8] This allows for rough estimates of number of detected barcodes (nuclei/cells) that pass filtering settings such as number of unique fragments per nucleus and fraction of fragments overlapping transcriptional start sites (TSS) (Figure 6E). The number of detected nuclei should be 50%–80% of the nuclei loaded in the 10× Chromium controller. Like bulk ATAC-seq the fragment size distribution of the filtered barcodes (Figure 6F) should be equivalent to the sequenced library.

LIMITATIONS

The main limitation of both bulk and snATAC-seq is the need for a clean nuclei preparation. When optimizing this protocol, we have put special emphasis on nuclei preparation and have devised two separate nuclei preparation schemes, one for bulk ATAC-seq and another for snATAC-seq. As detailed above, snATAC-seq is more laborious and more expensive compared to bulk ATAC-seq. However, in contrast to bulk ATAC-seq, it provides insights into heterogeneity within cell populations as well as cell type-specific data.

TROUBLESHOOTING

Problem 1

Step 8: No visible pellet after centrifugation.

Potential solution

This sometimes occurs in unhealthy liver (e.g., fatty liver) and almost always means that there are no nuclei to continue with. Repeat nuclei prep, preferably from a different liver. If this is not possible, use another piece from the same liver. Notice that after step 9 the pellet might be hard to visualize, that is fine.

Problem 2

Step 11: When counting nuclei in each sample, some samples show significantly lower nuclei count (by an order of magnitude).

Potential solution

Do not continue with the samples showing low nuclei count. Continuing with such samples is not ideal even if you have enough nuclei. This is because a low nuclei count is usually indicative of low nuclei quality. Repeat nuclei prep, preferably from a different liver. If this is not possible, use another piece from the same liver.

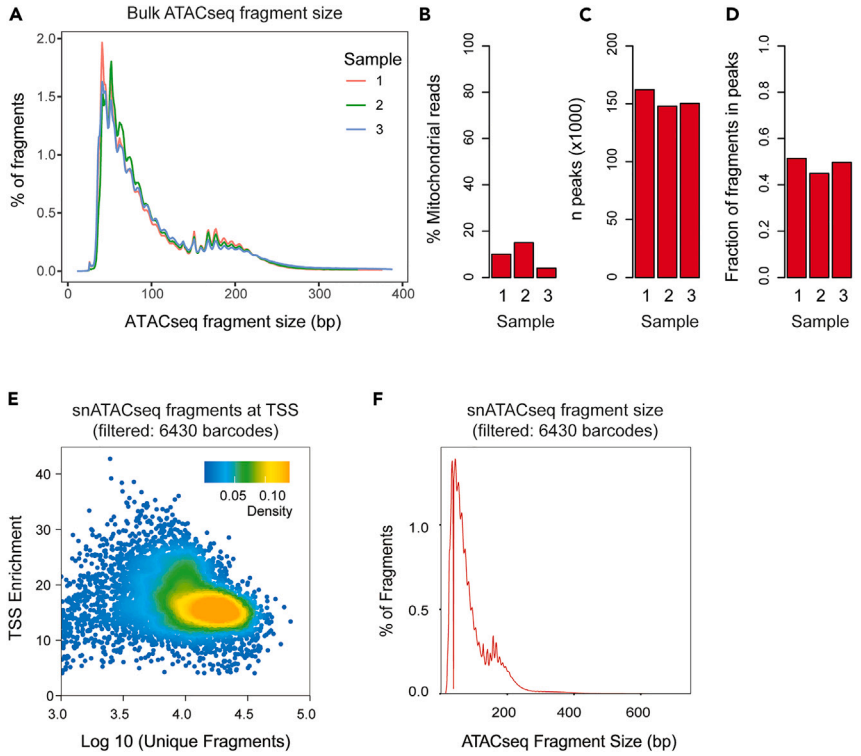


Figure 6. Quality control of sequenced bulk ATAC and snATAC libraries

(A–D) Distribution of post-sequencing bulk ATAC-seq fragment size (A), number of mitochondrial reads in the sequenced library (B), number of detected peaks (C) and fraction of reads in the detected peaks (D).

(E and F) Number of detected snATAC-seq fragments at TSS (E) in individual high-quality barcodes and distribution of snATAC-seq fragment size (F).

Problem 3

Step 20: The wrong index primer was added.

Potential solution

If you plan to pool your samples during sequencing and two samples contain the same index/barcode you will not be able to differentiate between reads originating from the two samples with identical index/barcode. Thus, you can use the leftover DNA (~10 μL) from step 18 and add the correct index to it.

Problem 4

Low amount of tagmented mono-nuclesomal DNA (>300nt, see explanation in Figure 5 legend) visible in the Tapestation output (Figure 5).

Potential solution

The DNA is either over-tagmented or the nuclei quality is compromised resulting in chromatin decompaction and/or leakage of genomic DNA. The Tn5-to-nuclei ratio can be adjusted to prevent over-tagmentation. Douncing can be optimized to retrieve better nuclei quality. Evaluating nuclei quality in step 8 (bulk ATAC) and step 55 (snATAC) during nuclei counting will provide some insights into nuclei quality. Pay specific attention to partially broken nuclei leaking chromatin content (Figure 4). We usually observe well below 10% partially broken nuclei. If a high degree of partially broken nuclei is observed, it is recommended to repeat the nuclei purification step.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ido Goldstein (ido.goldstein@mail.huji.ac.il).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate new code or data.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2023.102462.

ACKNOWLEDGMENTS

The methodology development in the Grøntved lab was supported by the ATLAS center of excellence supported by a grant from the Danish National Research Foundation (grant #141). Work in the Goldstein lab was supported by the European Research Council (ERC-StG grant number 947907), the Israel Science Foundation (ISF, grants numbers 1469/19, 3533/19), the Canadian Institutes of Health Research (CIHR), and the International Development Research Centre (IDRC). We would like to thank Dr. Abed Nasereddin and Dr. Idit Shiff from the Genomic Applications Lab, Hebrew University of Jerusalem, and Ronni Nielsen from the Department of Biochemistry and Molecular Biology, University of Southern Denmark, for their invaluable help, support, and expertise in high-throughput sequencing.

AUTHOR CONTRIBUTIONS

N.K. and N.I.T. developed and optimized the method and wrote the manuscript. T.V.D. and M.C-N. developed and optimized the method. L.G. and I.G. developed the method, acquired funding, supervised the project, and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Corces, M.R., Trevino, A.E., Hamilton, E.G., Greenside, P.G., Sinnott-Armstrong, N.A., Vesuna, S., Satpathy, A.T., Rubin, A.J., Montine, K.S., Wu, B., et al. (2017). An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat. Methods *14*, 959–962. https://doi.org/10.1038/nmeth.4396.

2. Grandi, F.C., Modi, H., Kampman, L., and Corces, M.R. (2022). Chromatin accessibility profiling by ATAC-seq. Nat. Protoc. *17*, 1518–1552. https://doi.org/10.1038/s41596-022-00692-9.

3. Buenrostro, J.D., Giresi, P.G., Zaba, L.C., Chang, H.Y., and Greenleaf, W.J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat. Methods *10*, 1213–1218. https://doi.org/10.1038/nmeth.2688.

4. Buenrostro, J.D., Wu, B., Chang, H.Y., and Greenleaf, W.J. (2015). ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. Curr. Protoc. Mol. Biol. *109*. 21-29.21-21.29.29. https://doi.org/10.1002/0471142727.mb2129s109.

5. Shi, Z., Das, S., Morabito, S., Miyoshi, E., and Swarup, V. (2022). Protocol for single-nucleus ATAC sequencing and bioinformatic analysis in frozen human brain tissue. STAR Protoc. *3*, 101491. https://doi.org/10.1016/j.xpro.2022.101491.

6. Rickner, H.D., Niu, S.Y., and Cheng, C.S. (2019). ATAC-seq Assay with Low Mitochondrial DNA Contamination from Primary Human CD4+ T Lymphocytes. J. Vis. Exp. https://doi.org/10.3791/59120.

7. Zhang, Y., Liu, T., Meyer, C.A., Eeckhoute, J., Johnson, D.S., Bernstein, B.E., Nusbaum, C., Myers, R.M., Brown, M., Li, W., and Liu, X.S. (2008). Model-based analysis of ChIP-Seq (MACS). Genome Biol. *9*, R137. https://doi.org/10.1186/gb-2008-9-9-r137.

8. Granja, J.M., Corces, M.R., Pierce, S.E., Bagdatli, S.T., Choudhry, H., Chang, H.Y., and Greenleaf, W.J. (2021). ArchR is a scalable software package for integrative single-cell chromatin accessibility analysis. Nat. Genet. *53*, 403–411. https://doi.org/10.1038/s41588-021-00790-6.