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Ultra-deep ATAC-seq for sorted neurons

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We use this protocol and it's working

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Abstract

Isolation of neurons from frozen post-mortem human brain tissue and preparation of ATAC-seq libraries for ultra-deep sequencing and somatic mutation detection.

Protocol materials

Tagmentase (Unloaded) **Diagenode Catalog #**C01070010-20 Step 1

Tagmentation Buffer (2X) **Diagenode Catalog #**C01019043-5000 Step 1

Anti-NeuN Antibody, clone A60, Alexa Fluor®488 conjugated **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**MAB377X

Step 1

DAPI Solution (1 mg/mL) **Thermo Fisher Scientific Catalog #**62248 Step 1

DNA Clean & Concentrator-5 (capped) **Zymo Research Catalog #**D4014 Step 1

Nextera XT Index Kit v2 Set B (96 indexes, 384 samples) **Illumina, Inc. Catalog #**FC-131-2002 Step 1

NEBNext High-Fidelity 2X PCR Master Mix - 250 rxns **New England Biolabs Catalog #**M0541L Step 1

Isolation of Nuclei from Adult Human Brain Tissue (modified from Allen Human Brain Tissue PF0291)

1 Reagent list:

- Tagmentase (Unloaded) **Diagenode Catalog #**C01070010-20
- Tagmentation Buffer (2X) **Diagenode Catalog #**C01019043-5000
- Anti-NeuN Antibody, clone A60, Alexa Fluor®488 conjugated **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**MAB377X
- DAPI Solution (1 mg/mL) **Thermo Fisher Scientific Catalog #**62248
- DNA Clean & Concentrator-5 (capped) **Zymo Research Catalog #**D4014
- Nextera XT Index Kit v2 Set B (96 indexes, 384 samples) **Illumina, Inc. Catalog #**FC-131-2002
- NEBNext High-Fidelity 2X PCR Master Mix - 250 rxns **New England Biolabs Catalog #**M0541L

2 **Buffer preparation:** On the day prior to sorting, prepare the following buffers:
a. Nuclei Isolation Media (NIM):

	A	B	C	D	E
	Formula(in milliQ water)	Ingredients:	For 1.5 mL(1 ATAC-seq brain)	For 5 mL(3 ATAC-seq brains)	For 20 mL
	0.25M Sucrose	Sucrose	0.13g	0.43 g	1.72 g
	25mM KCl	2M KCl	18.8 µL	62.7 uL	250.8 uL
	5mM MgCl2	1M MgCl2	7.5 uL	25 uL	100 uL
	10mM Tris-HCl, pH 8	1M Tris-HCl, pH 8	15 uL	50 uL	200 uL
	0.1% Triton X-100	10% Triton X-100	15 uL	50 uL	200 uL
	Water	Water	~1410 uL	~4.7 mL	18.8 mL

Filter through 0.22µM filter and store at 4°C. Can keep for ~1-2 weeks.

b. Blocking buffer:

	A	B	C	D	E
	Formula (in milliQ water)	Ingredients:	For 5 mL	For 10 mL	For 20 mL
	Water	Water	4.3 mL	8.6 mL	17.2 mL
	1X PBS	10X PBS	500 uL	1000 uL	2000 uL
	0.8% BSA	20% BSA	200 uL	400 uL	800 uL

Store at 4°C. Can keep for ~1-2 weeks.

c. 1X Tagmentation buffer w/ BSA for ATAC-seq:

	A	B	C
	Ingredients:	For 4 ATAC-seq reactions	For 6 ATAC-seq reactions
	2X Tagmentation buffer (Diagenode)	220 uL	330 uL
	5% digitonin	0.88 uL	1.3 uL
	10% Tween-20	4.4 uL	6.6 uL
	PBS	145 uL	218 uL
	20% BSA	22 uL	33 uL
	Water	25.5 uL	38.3 uL

Place at 4°C until ready to use.

3 **Load Tn5:** Mix 1 µL of annealed oligo A/oligo Rev with 1 µL of the annealed oligo B/oligo Rev as per Diagenode instructions.

4 Add 2 µL of Diagenode Tagmentase

5 Incubate at RT for 30 min

6 Add 2 µL glycerol. Place at -20°C until ready for use. Can store for a few week.

7 **Tissue preparation:**
Chill tweezers, scalpel, and dissecting plate (cell culture plate) on dry ice. Chill dounce homogenizers with B pestle on ice. Make sure swinging-bucket centrifuge is cooled to 4°C.

8 Spray down all surfaces and pipettes with 70% Ethanol and DNA Away

9 Make homogenization buffer (NIM + additives): Homogenization buffer: 5mL NIM + 5 uL 1M DTT (1 mM final concentration) + Mini Protease inhibitor (1/2 tablet) + 25 uL 100 mM spermidine (0.5 mM final concentration).

10 Scrape ~10mg frozen brain tissue onto cell culture plate, either on top of dry ice or in a cryostat at -20°C. Add 1.5 mL Homogenization buffer to tissue. Add tissue/buffer mix to Dounce homogenizer.

11 Dounce 15-20 strokes with B pestle. If the tissue is large, use more homogenization buffer (we have used up to 5 mL) and dounce with A pestle followed by B pestle.

12 Filter through 40µM filter into Eppendorf tube.

13 Spin in 4°C swinging-bucket centrifuge for 10min @ 900xg

14 While spin is going on, clean homogenizers by rinsing thoroughly with ddH2O, and then soaking them in 20% bleach for at least 20 minutes.

15 Make immunostaining buffer (Blocking buffer + 1:1000 dilution NeuN-488).

16 Remove supernatant. Do not disturb the pellet.

17 Add 1mL Blocking buffer + NeuN. Resuspend pellet gently with pipette. Rotate end-to-end in the cold room for 15-20 minutes.

18 Spin in 4°C swinging-bucket centrifuge for 5min @ 400xg

19 Make DAPI solution. First, dilute stock DAPI (1mg/mL) 1:15 in blocking buffer. Then, add 1 µL of the diluted dapi solution to 1 mL blocking buffer.

20 Remove supernatant from pellet.

21 Add 1mL Blocking buffer + Dapi and again resuspend. Strain through 40 µM filter into new Eppendorf tube immediately before nuclei sorting.

22 Prepare tubes for ATAC-seq: Add 60 µL of 1X Tagmentation buffer into each tube for ATAC-seq (2 tubes per brain)

ATAC-seq (modified from Omni-ATAC protocol (Corces et al., 2017))

23 Sort 10,000 NeuN+ nuclei into 60µL of tagmentation buffer per ATAC-seq (2 replicates per brain sample). Proceed immediately after sorting to ATAC-seq

24 After sorting, spin each tube in 4 deg swinging bucket centrifuge at 500xg for 5 minutes.

25 Remove ~75 µL of supernatant leaving ~10 µL at bottom. Tap tube gently to mix pellet.

26 Add 45 µL of 1X Tagmentation buffer. Do not mix.

27 Add 1 µL of loaded Tn5. Pipette gently 3-4 times and tap to mix.

28 Incubate at 37°C for 30 minutes in the Thermomixer without mixing. Gently tap tube at ~10 minutes and at ~20 minutes into the incubation to mix.

29 Stop tagmentation reaction by adding 250 µL of DNA binding buffer from Zymo Clean and Concentrator 5 kit. Add to sample tube, pipette 6x or vortex until homogenous.

30 Follow Zymo kit instructions to purify DNA:
1. Add solution to spin-column tube. Spin at 10,000xg for 30 seconds.
2. Wash x 200 µL wash buffer. Spin at 10,000xg for 30 seconds.
3. Repeat wash x 200 µL wash buffer. Spin at 10,000xg for 30 seconds.
4. Transfer spin-column tube into Eppendorf tube. Add 20 µL of water. Let sit for 1 minute. Then spin at 10,000xg for 1 minute.

31 Prepare PCR reaction on ice:
- 2.5 µL i5 primer
- 2.5 µL i7 primer
- 25 µL NEBNext High-Fidelity 2X PCR Master Mix, thaw on ice and invert until homogenous
- 20 µL purified tagmented DNA

32 PCR amplification:
1. 72°C x 5 minutes
2. 98°C x 30 seconds
3. 98°C x 10 seconds
4. 63°C x 30 seconds
5. 72°C x 1 min
6. Loop back to step [3] 8 times (9 cycles total)
7. 72°C x 1 minute
8. 4°C hold

33 Clean the library using Zymo clean and concentrator 5 kit. Add 250 µL of DNA binding buffer to PCR reaction and follow Zymo kit directions to purify.

34 Elute in 40-50 µL 0.1X TE (or Zymo elution buffer). Let sit for 1 minute, spin for 1 minute.

35 Run a 1:4 dilution of library on HS5000 Tapestation

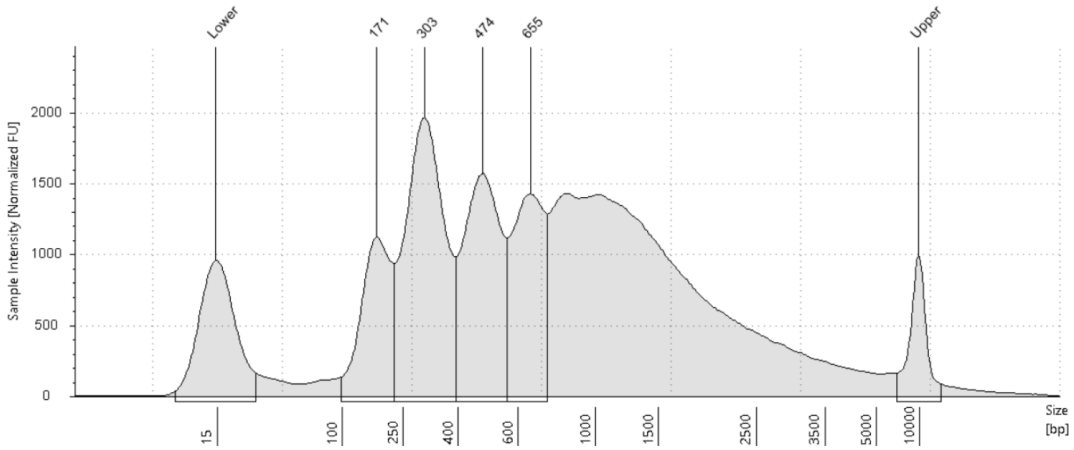
36

Expected result

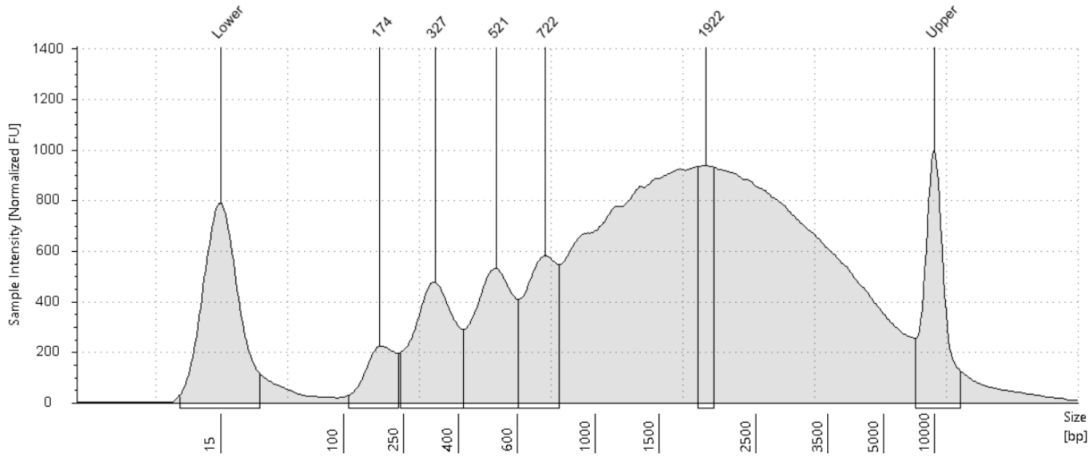
Library traces from frozen brain tissues are quite variable. In general, a nucleosomal ladder should be apparent with a sub-nucleosomal peak around ~170bp (keep in mind the size of the adapters is around ~100bp, so this corresponds to an insert size of ~70bp), mono-nucleosomal peak around ~300bp, di-nucleosomal peak around ~450bp, tri-nucleosomal peak around ~600bp and so on.

The strength of the nucleosome ladder relative to larger molecular weight fragments can differ greatly between samples (and even between replicates). We have had success sequencing ATAC libraries with traces seen below. Note that for brain samples, the mono-nucleosomal peak should be larger than the sub-nucleosomal peak. An overly large sub-nucleosomal peak can indicate degradation of chromatin structure

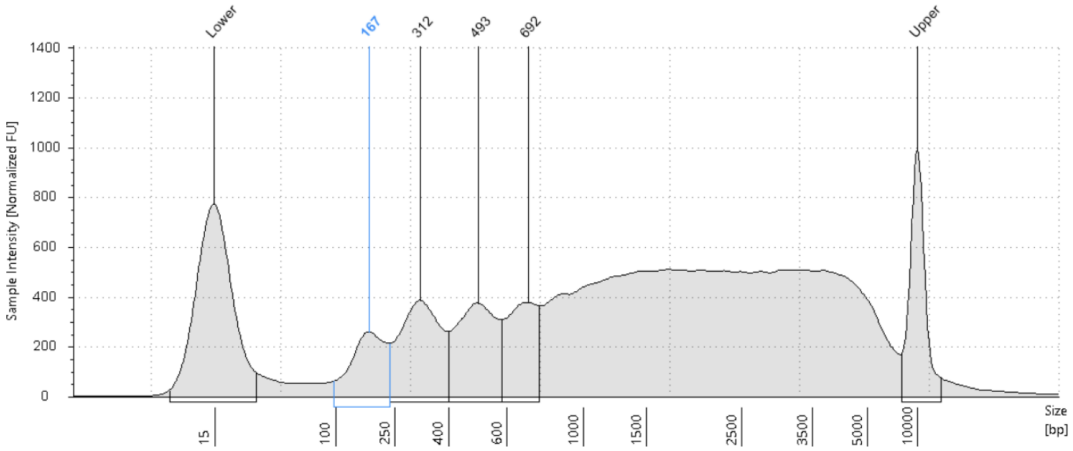
Below are three representative traces of libraries we have successfully sequenced:



ATAC library with very apparent nucleosomal ladder



ATAC library with a greater quantity of large molecular weight fragments relative to nucleosomal ladder



ATAC library with a nucleosomal ladder as well as a comparable quantity of large molecular weight fragments

37 Store libraries at -20°C.

Protocol references

CITATION

Corces MR, Trevino AE, Hamilton EG, Greenside PG, Sinnott-Armstrong NA, Vesuna S, Satpathy AT, Rubin AJ, Montine KS, Wu B, Kathiria A, Cho SW, Mumbach MR, Carter AC, Kasowski M, Orloff LA, Risca VI, Kundaje A, Khavari PA, Montine TJ, Greenleaf WJ, Chang HY (2017). An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues..
LINK
https://doi.org/10.1038/nmeth.4396

CITATION

LeinU01 BRAIN grant. Isolation of Nuclei from Adult Human Brain Tissue for 10x Genomics Platform. protocols.io.
LINK
https://protocols.io/view/isolation-of-nuclei-from-adult-human-brain-tissue-y6rfzd6

CITATION

Matevossian A, Akbarian S (2008). Neuronal nuclei isolation from human postmortem brain tissue..
LINK
https://doi.org/pii:914.10.3791/914

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Corces MR, Trevino AE, Hamilton EG, Greenside PG, Sinnott-Armstrong NA, Vesuna S, Satpathy AT, Rubin AJ, Montine KS, Wu B, Kathiria A, Cho SW, Mumbach MR, Carter AC, Kasowski M, Orloff LA, Risca VI, Kundaje A, Khavari PA, Montine TJ, Greenleaf WJ, Chang HY. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues.
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Matevossian A, Akbarian S. Neuronal nuclei isolation from human postmortem brain tissue.
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