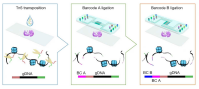
Oct 22, 2024

Yale University_Spatial ATAC Sequencing for Fixed Fresh Frozen Human Lymph node Tissue via DBiT-seq



Forked from Spatial Multi-omics Sequencing for Fixed Tissue via DBiT-seq

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Negin Farzad[1], Yanxiang Deng[1], Archibald Enninful[1], yao.lu.yl[1], Rong Fan[1]

[1]Yale University

Cellular Senescence Net…

Negin Farzad
Yale University

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Farzad N, Enninful A, Bao S, Zhang D, Deng Y, Fan R. Spatially resolved epigenome sequencing via Tn5 transposition and deterministic DNA barcoding in tissue. Nat Protoc. 2024 Jun 28. doi: 10.1038/s41596-024-01013-y. Epub ahead of print. PMID: 38943021.

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We use this protocol and it's working

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Abstract

This protocol describes the use of Deterministic Barcoding in Tissue for spatial ATAC sequencing to construct an epigenome atlas of fixed fresh frozen human lymph node tissue samples. This approach uses a microfluidic-based method to introduce combinatorial DNA oligo barcodes directly to the cells in a tissue section fixed on a glass slide. This method spatially resolved genome-wide profiling of transposase-accessible chromatin sequencing for chromatin accessibility.This technique does not directly resolve single cells but can achieve a near-single-cell resolution for spatial epigenomic analysis.

Materials

KEY RESOURCES TABLE

	A	B	C
	Name	**Catalog number**	**Vender**
	Formaldehyde solution	PI28906	Thermo Fisher Scientific
	Glycine	50046	Sigma-Aldrich
	NaCl	AM9760G	Thermo Fisher Scientific
	Digitonin	G9441	Promega
	MgCl2	AM9530G	Thermo Fisher Scientific
	NP40	11332473001	Sigma-Aldrich
	EDTA Solution pH 8.0	AB00502	AmericanBio
	Bovine Serum Albumin (BSA)	A8806	Sigma-Aldrich
	Triton X-100	T8787	Sigma-Aldrich
	T4 DNA Ligase	M0202L	New England Biolabs
	T4 DNA Ligase Reaction Buffer	B0202S	New England Biolabs
	NEBuffer 3.1	B7203S	New England Biolabs
	DPBS	14190144	Thermo Fisher Scientific
	Proteinase K	EO0491	Thermo Fisher Scientific
	Ampure XP beads	A63880	Beckman Coulter
	NEBNext High-Fidelity 2X PCR Master Mix	M0541L	New England Biolabs
	SYBR Green I Nucleic Acid Gel Stain	S7563	Thermo Fisher Scientific

	A	B	C
	DNA Clean & Concentrator-5	D4014	Zymo Research
	Tn5 Transposase - unloaded	C01070010	Diagenode
	Tagmentation Buffer (2x)	C01019043	Diagenode
	Sodium dodecyl sulfate	71736	Sigma-Aldrich
	Maxima H Minus Reverse Transcriptase (200 U/L)	EP0751	Thermo Fisher Scientific
	dNTP mix	R0192	Thermo Fisher Scientific
	SUPERased In RNase Inhibitor	AM2694	Thermo Fisher Scientific
	Ampure XP beads	A63880	Beckman Coulter
	Dynabeads MyOne C1	65001	Thermo Fisher Scientific
	RNase Inhibitor	Y9240L	Enzymatics
	Kapa Hotstart HiFi ReadyMix	KK2601	Kapa Biosystems
	Nextera XT DNA Preparation Kit	FC-131-1024	Illumina

Table S1A. **List of reagents**

	Oligo Name	Sequence
	Ligation Linker	CGAATGCTCTGGCCTCTCAAGCACGTGGAT
	P5 oligo	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
	P7 oligo (701)	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
	P7 oligo (702)	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
	P7 oligo (703)	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
	P7 oligo (704)	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
	P7 oligo (705)	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
	P7 oligo (706)	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT

Table S2A. **List of PCR Oligo.**

	A	B
	Barcode A-1	/5Phos/AGGCCAGAGCATTCGAACGTGATTTTTTTTTTTTTTTTVN
	Barcode A-2	/5Phos/AGGCCAGAGCATTCGAAACATCGTTTTTTTTTTTTTTTTVN
	Barcode A-3	/5Phos/AGGCCAGAGCATTCGATGCCTAATTTTTTTTTTTTTTTTVN
	Barcode A-4	/5Phos/AGGCCAGAGCATTCGAGTGGTCATTTTTTTTTTTTTTTTVN
	Barcode A-5	/5Phos/AGGCCAGAGCATTCGACCACTGTTTTTTTTTTTTTTTTVN
	Barcode A-6	/5Phos/AGGCCAGAGCATTCGACATTGGCTTTTTTTTTTTTTTTTVN
	Barcode A-7	/5Phos/AGGCCAGAGCATTCGCAGATCTGTTTTTTTTTTTTTTTTVN
	Barcode A-8	/5Phos/AGGCCAGAGCATTCGCATCAAGTTTTTTTTTTTTTTTTVN
	Barcode A-9	/5Phos/AGGCCAGAGCATTCGCGCTGATCTTTTTTTTTTTTTTTTVN
	Barcode A-10	/5Phos/AGGCCAGAGCATTCGACAAGCTATTTTTTTTTTTTTTTTVN
	Barcode A-11	/5Phos/AGGCCAGAGCATTCGCTGTAGCCTTTTTTTTTTTTTTTTVN
	Barcode A-12	/5Phos/AGGCCAGAGCATTCGAGTACAAGTTTTTTTTTTTTTTTTVN
	Barcode A-13	/5Phos/AGGCCAGAGCATTCGAACAACCATTTTTTTTTTTTTTTTVN
	Barcode A-14	/5Phos/AGGCCAGAGCATTCGAACCGAGATTTTTTTTTTTTTTTTVN
	Barcode A-15	/5Phos/AGGCCAGAGCATTCGAACGCTTATTTTTTTTTTTTTTTVN
	Barcode A-16	/5Phos/AGGCCAGAGCATTCGAAGACGGATTTTTTTTTTTTTTTTVN
	Barcode A-17	/5Phos/AGGCCAGAGCATTCGAAGGTACATTTTTTTTTTTTTTTTVN
	Barcode A-18	/5Phos/AGGCCAGAGCATTCGACACAGAATTTTTTTTTTTTTTTTVN
	Barcode A-19	/5Phos/AGGCCAGAGCATTCGACAGCAGATTTTTTTTTTTTTTTTVN
	Barcode A-20	/5Phos/AGGCCAGAGCATTCGACCTCCAATTTTTTTTTTTTTTTTVN
	Barcode A-21	/5Phos/AGGCCAGAGCATTCGACGCTCGATTTTTTTTTTTTTTTTVN
	Barcode A-22	/5Phos/AGGCCAGAGCATTCGACGTATCATTTTTTTTTTTTTTTTVN
	Barcode A-23	/5Phos/AGGCCAGAGCATTCGACTATGCATTTTTTTTTTTTTTTTVN
	Barcode A-24	/5Phos/AGGCCAGAGCATTCGAGAGTCAATTTTTTTTTTTTTTTTVN
	Barcode A-25	/5Phos/AGGCCAGAGCATTCGAGATCGCATTTTTTTTTTTTTTTTVN
	Barcode A-26	/5Phos/AGGCCAGAGCATTCGAGCAGGAATTTTTTTTTTTTTTTTVN
	Barcode A-27	/5Phos/AGGCCAGAGCATTCGAGTCACTATTTTTTTTTTTTTTTTVN
	Barcode A-28	/5Phos/AGGCCAGAGCATTCGATCCTGTATTTTTTTTTTTTTTTTVN
	Barcode A-29	/5Phos/AGGCCAGAGCATTCGATTGAGGATTTTTTTTTTTTTTTTVN
	Barcode A-30	/5Phos/AGGCCAGAGCATTCGCAACCACATTTTTTTTTTTTTTTTVN
	Barcode A-31	/5Phos/AGGCCAGAGCATTCGGACTAGTATTTTTTTTTTTTTTTTVN
	Barcode A-32	/5Phos/AGGCCAGAGCATTCGCAATGGAATTTTTTTTTTTTTTTTVN
	Barcode A-33	/5Phos/AGGCCAGAGCATTCGCACTTCGATTTTTTTTTTTTTTTTVN
	Barcode A-34	/5Phos/AGGCCAGAGCATTCGCAGCGTTATTTTTTTTTTTTTTTTVN

	A	B
	Barcode A-35	/5Phos/AGGCCAGAGCATTCGCATACCAATTTTTTTTTTTTTTTVN
	Barcode A-36	/5Phos/AGGCCAGAGCATTCGCCAGTTCATTTTTTTTTTTTTTTVN
	Barcode A-37	/5Phos/AGGCCAGAGCATTCGCCGAAGTATTTTTTTTTTTTTTTVN
	Barcode A-38	/5Phos/AGGCCAGAGCATTCGCCGTGAGATTTTTTTTTTTTTTTVN
	Barcode A-39	/5Phos/AGGCCAGAGCATTCGCCTCCTGATTTTTTTTTTTTTTTVN
	Barcode A-40	/5Phos/AGGCCAGAGCATTCGCGAACTTATTTTTTTTTTTTTTTVN
	Barcode A-41	/5Phos/AGGCCAGAGCATTCGCGACTGGATTTTTTTTTTTTTTTVN
	Barcode A-42	/5Phos/AGGCCAGAGCATTCGCGCATACATTTTTTTTTTTTTTTVN
	Barcode A-43	/5Phos/AGGCCAGAGCATTCGCTCAATGATTTTTTTTTTTTTTTVN
	Barcode A-44	/5Phos/AGGCCAGAGCATTCGCTGAGCCATTTTTTTTTTTTTTTVN
	Barcode A-45	/5Phos/AGGCCAGAGCATTCGCTGGCATATTTTTTTTTTTTTTTVN
	Barcode A-46	/5Phos/AGGCCAGAGCATTCGGAATCTGATTTTTTTTTTTTTTTVN
	Barcode A-47	/5Phos/AGGCCAGAGCATTCGCAAGACTATTTTTTTTTTTTTTTVN
	Barcode A-48	/5Phos/AGGCCAGAGCATTCGGAGCTGAATTTTTTTTTTTTTTTVN
	Barcode A-49	/5Phos/AGGCCAGAGCATTCGGATAGACATTTTTTTTTTTTTTTVN
	Barcode A-50	/5Phos/AGGCCAGAGCATTCGGCCACATATTTTTTTTTTTTTTTVN

Table S2B. List of DNA Barcode A Sequences.

	A	B
	Barcode B-1	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAACGTGATATCCACGTGCTTGAG
	Barcode B-2	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAAACATCGATCCACGTGCTTGAG
	Barcode B-3	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNATGCCTAAATCCACGTGCTTGAG
	Barcode B-4	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAGTGGTCAATCCACGTGCTTGAG
	Barcode B-5	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACCACTGTATCCACGTGCTTGAG
	Barcode B-6	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACATTGGCATCCACGTGCTTGAG
	Barcode B-7	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCAGATCTGATCCACGTGCTTGAG
	Barcode B-8	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCATCAAGTATCCACGTGCTTGAG
	Barcode B-9	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCGCTGATCATCCACGTGCTTGAG
	Barcode B-10	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACAAGCTAATCCACGTGCTTGAG

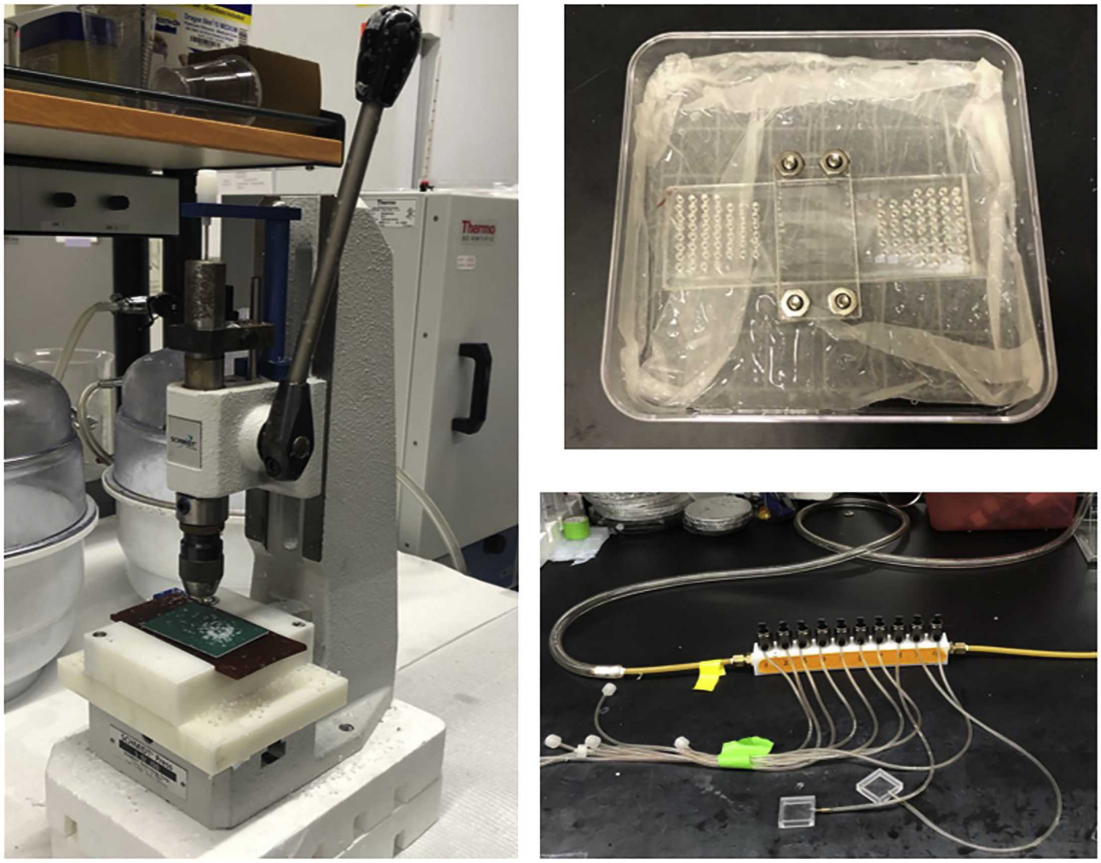
	A	B
	Barcode B-11	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCTGTAGCCATCCACGTGCTTGAG
	Barcode B-12	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAGTACAAGATCCACGTGCTTGAG
	Barcode B-13	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAACAACCAATCCACGTGCTTGAG
	Barcode B-14	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAACCGAGAATCCACGTGCTTGAG
	Barcode B-15	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAACGCTTAATCCACGTGCTTGAG
	Barcode B-16	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAAGACGGAATCCACGTGCTTGAG
	Barcode B-17	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAAGGTACAATCCACGTGCTTGAG
	Barcode B-18	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACACAGAAATCCACGTGCTTGAG
	Barcode B-19	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACAGCAGAATCCACGTGCTTGAG
	Barcode B-20	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACCTCCAAATCCACGTGCTTGAG
	Barcode B-21	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACGCTCGAATCCACGTGCTTGAG
	Barcode B-22	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACGTATCAATCCACGTGCTTGAG
	Barcode B-23	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNACTATGCAATCCACGTGCTTGAG
	Barcode B-24	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAGAGTCAAATCCACGTGCTTGAG
	Barcode B-25	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAGATCGCAATCCACGTGCTTGAG
	Barcode B-26	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAGCAGGAAATCCACGTGCTTGAG
	Barcode B-27	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNAGTCACTAATCCACGTGCTTGAG
	Barcode B-28	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNATCCTGTAATCCACGTGCTTGAG
	Barcode B-29	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNATTGAGGAATCCACGTGCTTGAG
	Barcode B-30	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCAACCACAATCCACGTGCTTGAG
	Barcode B-31	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNGACTAGTAATCCACGTGCTTGAG
	Barcode B-32	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCAATGGAAATCCACGTGCTTGAG
	Barcode B-33	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCACTTCGAATCCACGTGCTTGAG

	A	B
	Barcode B-34	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCAGCGTTAATCCACGTGCTTGAG
	Barcode B-35	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCATACCAAATCCACGTGCTTGAG
	Barcode B-36	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCCAGTTCAATCCACGTGCTTGAG
	Barcode B-37	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCCGAAGTAATCCACGTGCTTGAG
	Barcode B-38	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCCGTGAGAATCCACGTGCTTGAG
	Barcode B-39	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCCTCCTGAATCCACGTGCTTGAG
	Barcode B-40	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCGAACTTAATCCACGTGCTTGAG
	Barcode B-41	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCGACTGGAATCCACGTGCTTGAG
	Barcode B-42	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCGCATACAATCCACGTGCTTGAG
	Barcode B-43	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCTCAATGAATCCACGTGCTTGAG
	Barcode B-44	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCTGAGCCAATCCACGTGCTTGAG
	Barcode B-45	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCTGGCATAATCCACGTGCTTGAG
	Barcode B-46	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNGAATCTGAATCCACGTGCTTGAG
	Barcode B-47	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNCAAGACTAATCCACGTGCTTGAG
	Barcode B-48	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNGAGCTGAAATCCACGTGCTTGAG
	Barcode B-49	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNGATAGACAATCCACGTGCTTGAG
	Barcode B-50	/5Biosg/CAAGCGTTGGCTTCTCGCATCTNNNNNNNNNNGCCACATAATCCACGTGCTTGAG

Table S2C. List of DNA Barcode B Sequences.

- This protocol requires using a microfluidic device fabricated with PDMS using soft lithography.
- A hole punching machine (SCHMIDT® Manual Press) is needed to punch the inlet and outlet holes in the PDMS chip after its fabrication.

- The tissue of interest should be placed on the center of a poly-L-lysine coated glass slide. (CatLog no. 63478-AS, electron microscopy sciences).
- A custom-designed acrylic clamp with screws is needed to hold the PDMS device and the glass slide together firmly.
- The silicon wafer used for fabricating the PDMS mold is purchased from WaferPro (CatLog. No. C04004).
- The photoresist is purchased from MicroChem Laboratory (CatLog. No. SU-8 2010).
- A homemade laboratory vacuum system(See Figure Below) is needed to applying suction to move fluid in the microfluidic channels.
- Microscopy – The tissue of interest can be scanned and imaged using EVOS (Thermo Fisher EVOS fl), typically at a magnification of 10×. Any suitable optical microscope can be used.
- A Laser Engraver Cutter is used to cut out the acrylic barcode and lysis clamps.
- A “humidified chamber” (See Figure Below) to prevent reagent evaporation during incubation.



Key equipment used in DBiT-seq

Hole punch machine, the key device for DBiT-seq, and the house vacuum lines. (Left) SCHMIDT® Manual Press used for hole punching 2-mm diameter holes for the inlets and outlets of Chip-A and Chip-B. (Top Right) Example of a “humidified chamber” to prevent reagent evaporation during the incubation steps. (Bottom Right) House vacuum used for pulling reagents through the microchannels.

Recipes

Critical: Prepare the RT mix, template switch mix, ligation mix, PCR mix, 1X lysis solution, flow wash buffer, PBS-RI, 80% ethanol on the same day as usage. 2X lysis buffer, 1X B&W Buffer with 0.05% Tween-20, and 2X B&W Buffer can be stored for up to 6 months at room temperature.

PBS-RI

Reagent	Volume (µL)
1X PBS	5000
RNase Inhibitor (40 U/µL) (Enzymatics)	7
Total	**5007**

80% Ethanol

Reagent	Volume(ml)
RNase-free water	1

Reagent	Volume(ml)
100% Ethanol	4
Total	**5**

RT Mixture

Reagent	Volume (µL)
5X Maxima RT buffer	50
RNase-free water	32.8
RNase Inhibitor (Enzymatics)	1.6
Superase In RNase Inhibitor (Ambion)	3.2
dNTPs (10 mM stock)	12.5
Maxima H Minus Reverse Transcriptase	25
PBS-RI	100
Total	**225.1**

Ligation Mix

Reagent	Volume (µL)
10X T4 Ligase Buffer	27
1X NEB buffer 3.1 with 1% RI (Enzymatics)	115.8
5% Triton-X100	5.4
RNase Inhibitor (Enzymatics)	2.2
RNase-free water	69.5
SuperaseIn RNase Inhibitor (Ambion)	0.7
T4 DNA Ligase (400 U/µL)	11
Total	**231.6**

Template switch mix

Reagent	Volume (µL)
20% Ficoll PM-400	44
5X Maxima RT buffer	44
dNTPs (10 mM stock)	22
RNase Inhibitor (Enzymatics)	5.5
Maxima H Minus Reverse Transcriptase	11
Template Switch Primer (100 µM stock)	5.5
RNase-free water	88
Total	**220**

PCR mix

Reagent	Volume (µL)
2X Kapa Hifi HotStart master mix	110
Primer1 BC_0062 (10µM)	8.8
Primer2 BC_0108 (10µM)	8.8
RNase-free water	92.4
Total	**220**

Flow Wash Buffer

Reagent	Volume (mL)
1X PBS	4
10 % Triton X-100	0.04
Superase In RNase Inhibitor	0.01
Total volume	**4.05**

1X Lysis Solution

Reagent	Volume (µL)
1X PBS	50
2X Lysis Buffer	50
Proteinase K (20mg/mL) (Thermo)	10
Total	**110**

2X Lysis Buffer

Reagent	Stock Concentration	Final Concentration (2X)	Volume (mL)
Tris, pH 8.0	1 M	20 mM	0.5
NaCl	5 M	400 mM	2
EDTA, pH 8.0	0.5 M	100 mM	5
SDS	10%	4.4 %	11
RNase-free Water	NA	NA	6.5
Final Volume			**25**

1X B&W Buffer with 0.05% Tween-20

Reagent	Volume
1M Tris-HCl pH 8.0	100 µL
EDTA, 0.5M	20 µL
5M NaCl	4 mL
Tween 20 10%	100 µL
RNase-free water	15.78 mL
Total	**20 mL**

2X B&W Buffer

Reagent	Volume
1M Tris-HCl pH 8.0	500 µL
EDTA, 0.5M	100 µL
5M NaCl	20 mL
RNase-free water	29.4 mL
Total	**50 mL**

CRITICAL: Handle all in an RNase-free area.

Before start

The protocol below describes the reagents, equipment, and specific experimental steps for using the spatial-ATAC-seq platform on fixed fresh frozen tissue slides. The 20 μm microfluidic channel width were designed and validated to provide spatial epigenomic profiles on human lymph note tissue sections.

CRITICAL: Work in an RNase-free environment when the microfluidic device is not on the tissue slide. Use RNaseZap^{TM} or other commercially available cleaner solution and filter-tips. Clean surfaces and gloves with RNaseZap^{TM}.

CRITICAL: Keep reagents on ice at all times.

Fabricating the Silicon Wafer Device Mold

12h

1 Prepare chrome photomasks of the microfluidics chip by printing the high-resolution CAD files onto a glass substrate (Figure 1).

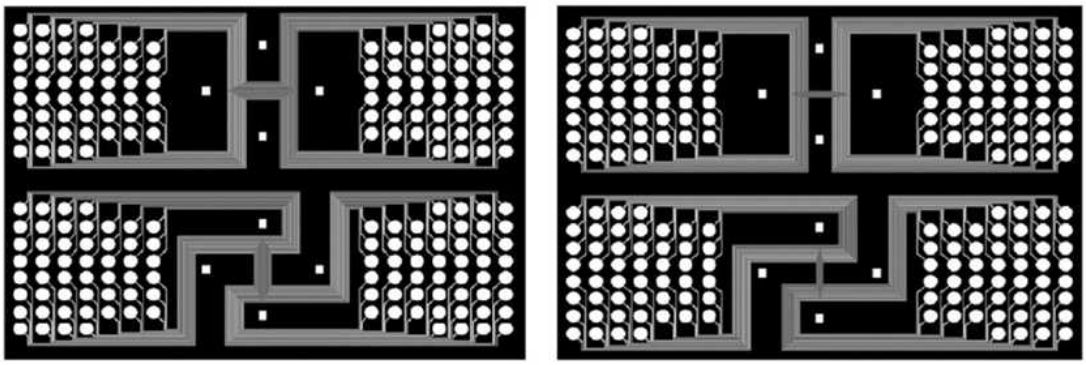


Figure 1: Microfluidic Photomask Design.

(Left) AutoCAD design for the 20 micron-width fifty channel Chip-A and Chip-B. (Right) AutoCAD design for 10 micron-width channel Chip-A and Chip-B.

NOTE: We have outsourced this step (Front Range Photomask, USA).

Using these masks, prepare a replica mold as follows:

2 Start the process by cleaning a 4-inch silicon wafer with 100% acetone (Aldrich) and then 100% isopropanol (Aldrich), then dry with compressed air.

NOTE: Acetone and isopropanol are mildly toxic. Use proper PPE when handling and discard waste in the appropriate containers.

3 Prepare a high-resolution computer-aided-design (CAD) file with the desired microfluidics chip design. A CAD file is also available in the link provided. (https://ars.els-cdn.com/content/image/1-s2.0-S2666166721002392-mmc4.zip)

4 Bake the wafer at 180°C for 10 min on a hot plate to dry it out.

5 Use a spin coater to evenly spread SU-8 2010 photoresist (MicroChem) for 10 µm device or SU-8 2025 (MicroChem) for 25 µm and 50 µm device onto the wafer at 500 rpm for 5 seconds followed by 1100 rpm (10 µm device), 3500 rpm (25 µm device) or 1750 rpm (50 µm device) for 40 seconds.

6 Soft bake the wafer for 3 min at 65°C (25 µm and 50 µm device) and 4 min (10 µm device) or 6 min (25 µm and 50 µm device) at 95°C.

7 Expose the SU-8 on the wafer through the photomask using Mask Aligner with a dose of 150 mJ/cm2 UV.

8 For the post exposure bake, bake the wafer for 1 min at 65°C (25 µm and 50 µm device) and 5 min (10 µm and 25 µm device) or 6 min (50 µm device) at 95°C.

9 Develop the SU-8 for 4 min (10 µm and 25 µm device) or 5 min (50 µm device) in a bath of ~50 mL SU-8 developer (MicroChem).

10 Rinse the wafer with 100% isopropanol and dry with compressed air.

11 Perform a hard bake by baking the wafer for 10 min at 180°C.

CRITICAL: This process needs to be carried out in a microelectronics cleanroom.

Pause Point: The replica mold can now be stored and reused indefinitely.

Creating the Acrylic Clamps

30m

12 Prepare the pattern and dimensions for the barcoding clamp and lysis clamp.

13 Peel covering of acrylic sheet and place it in the laser cutting machine.

14 Select the program dimensions and cut two pieces for the top and bottom of the barcoding clamp.

15 Place the top blank piece into the laser cutter and select the pattern to cut out the four holes in the corner for the screws and nuts.

16 Remove cut-out scraps with a pipet tip or a similar pointed tool.

17 Repeat steps 15 and 16 for the bottom blank piece.

18 Repeat steps 13 to 17 for the lysis clamp using the correct dimensions and pattern with an additional hole in the center of the top piece (Example shown in Figure 2).

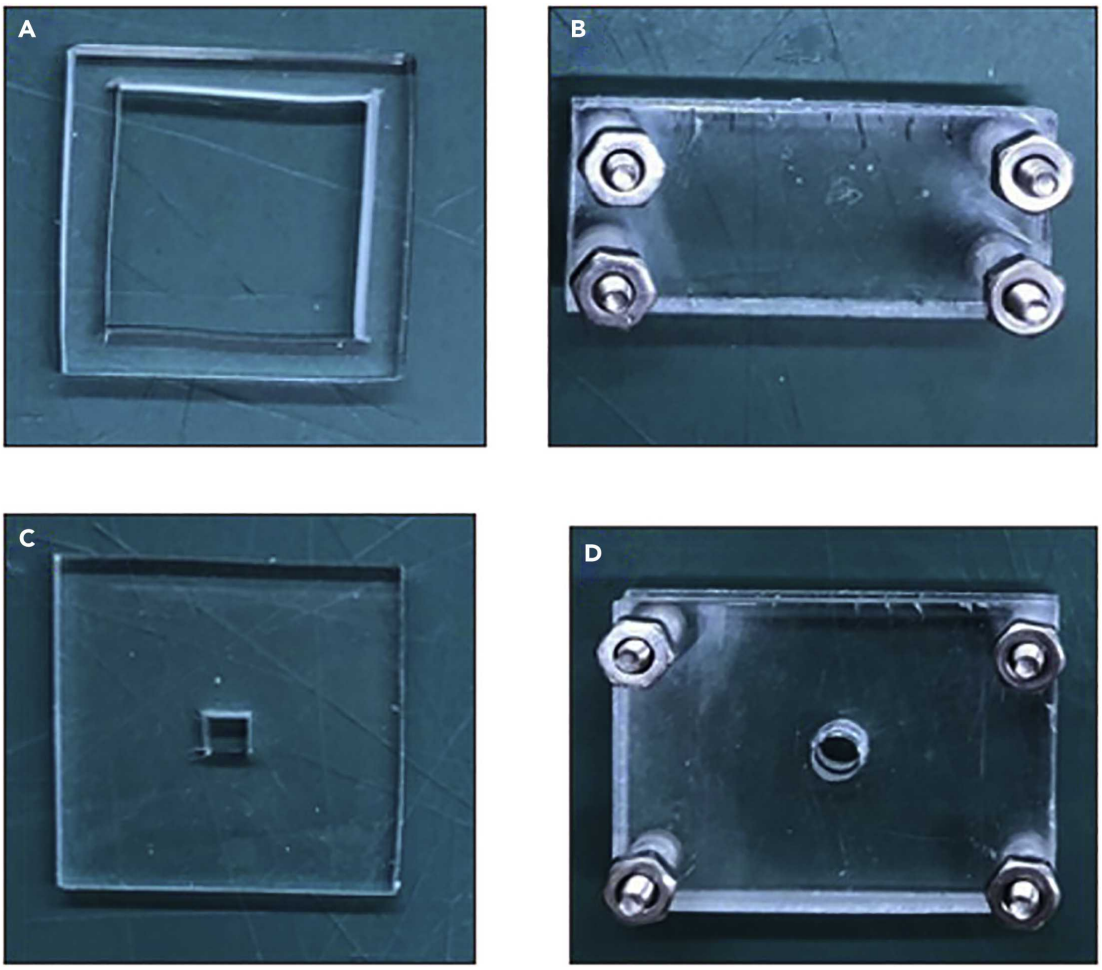


Figure 2. Key parts for setup. PDMS reservoirs and acrylic clamps used in DBiT-seq. (A) Inlet Reservoir. (B) Barcoding Clamp. (C) Lysis Reservoir. (D) Lysis Clamp.

NOTE: The acrylic clamps can be reused indefinitely.

Preparing the Microfluidic Device and reagent reservoirs

3h

19 Thoroughly mix polydimethylsiloxane (PDMS) elastomer base and curing agent (these come together) at a 10:1 ratio (See Figure 3).

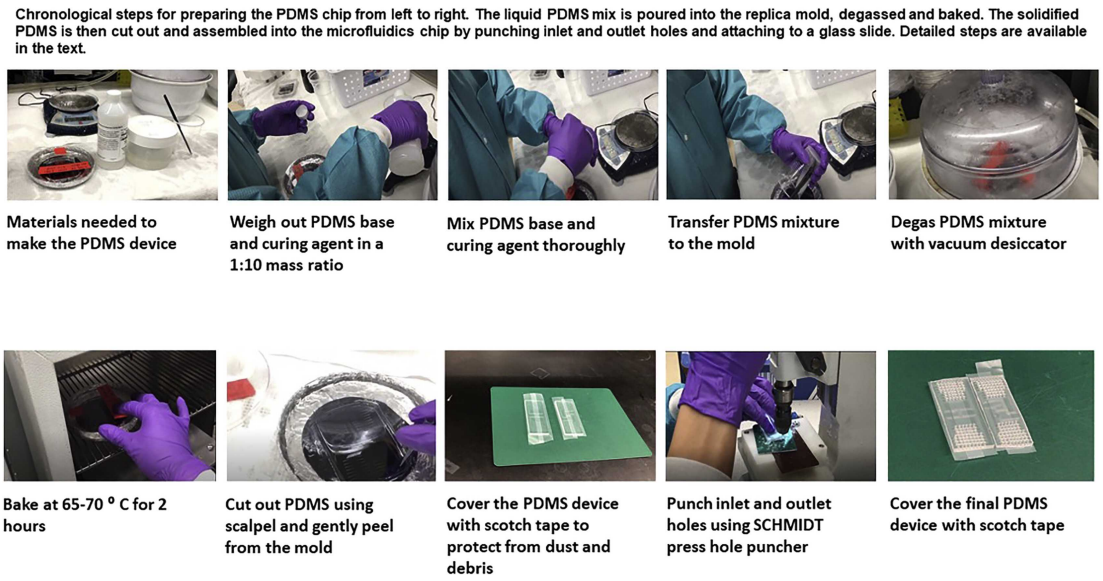


Figure 3: Step-by-step visual guide for making Chip-A and Chip-B.

20 Pour the mixture into the silicon device mold.

NOTE: Aim for a chip height of about 5mm.

21 Place in a vacuum desiccator until all bubbles dissipate from the mixture about 30-60 min.

22 Cure in an oven at 65-70˚C for a minimum of 2 hours and up to overnight.

NOTE: Make sure to place on an even surface to prevent uneven curing!

23 Cut out the cured device and hole punch each of the 50 inlets and outlets for the channels.

NOTE: Cut both Chip-A and Chip-B in the approximate size of a glass slide.
NOTE: Make sure there are no PDMS pieces left in the inlets or outlets.

24 Thoroughly clean the surface of the device with scotch tape.

25 Pour mixture in a container large enough to cut out two roughly 25x25x5mm PDMS pieces.

26 For the barcoding reservoir, cut out a 20x20mm piece in the center, barely large enough to surround the inlets of the chips when placed above.

27 For the lysis reservoir, depending on the device size used, cut out the center to barely surround the barcoded region of the tissue (1x1mm for the 10µm channel device, 2.5x2.5mm for the 25µm channel device).

NOTE: The reservoirs are reusable. Thoroughly wash with 70% ethanol after each use.

CRITICAL: Be careful when cutting out the Chips A and B. Make sure not to cut across any patterned areas of the silicon wafer mold, otherwise it will need to be replaced and re-fabricated.

Preparing Barcodes (A and B). 1h

28 Thoroughly mix the ligation linker 1 with each barcode $A_{1\text{-}50}$ at and ligation linker 2 with each barcode $B_{1\text{-}50}$ at a 1:1 ratio.

29 Place the 50 mixes in a thermal cycle and heat to 97˚C to anneal.

30 Slowly cool to room temperature at a rate of -0.1˚C/sec.

Store at -20˚C for up to 6 months.

Preparing the Tissue Slide 1d

31 25% sucrose overnight bath.

32 Embed in OCT frozen on dry ice.

33 Section at ~7 µm thickness for the 25µm channel device and ~5 µm thickness for the 10 µm channel device onto Poly-L-lysine slides.

34 Store slides at -80˚C for up to 6 months until use.

CRITICAL: Ensure that the tissue is sectioned as evenly as possible, otherwise the risk of cross-flow between the microfluidic channels will be increased. Tissue section should be placed at the center of a glass slide to the greatest extent.

Transposome assembly preparation

35

Prepare the transposome assembly annealing buffer (The Tn5 annealing buffer contains 400 µl of 1 M Tris-HCl pH 8.0, 100 µl of Nacl 5 M and 9.5 ml water. This annealing buffer is used to resuspend each oligos of Tn5-Rev, Tn5-A and Tn5-B in this annealing buffer to stock concentrations of 100 µM.

36 Resuspend the oligos (Tn5Erev, Tn5ME-A , and Tn5ME-B) in Annealing Buffer to stock concentration of 100 µM.

37 In a PCR tube, mix 10 µl of oligo Rev with 10 µl of oligo A.

38 In a separate PCR tube, mix 10 µl of oligo Rev with 10 µl of oligo B

39 Vortex the tubes and place them in a thermocycler, and run the thermocycler with the following program:

Temperature	Time
95 °C	5 min
Cool to 65 C	-0.1C/ second
65 °C	5 min
Cool to 4 °C	-0.1 C/second

The Annealed linker oligos can be stored at -20 C.

40 Mix 5 µl of each prepared oligo A/oligo Rev and oligo B/oligo Rev at the ratio of 1:1 in a pcr tube.

41 Add10 µl of unloaded Tn5 transposome (Diagnode Tagmentase) to the mixture of oligo A/oligo Rev and oligo B/oligo Rev.
After vortexing the mixture briefly, incubate the tube at 23 ºC for 30 minutes in a thermocycler.
The product can be use at the same day or stored in -20 ºC with 10 µl glycerol up to 60 days.

Tissue Preparation

1h

42 Remove stored sections from the freezer and allow to warm to room temperature for 10 minutes.

43 Clean sections by pipetting 2 mL of PBS-RI across the tissue.

44 If sections are not yet fixed, then fix here with 1 mL 0.2% PFA ,without methanol in 1X PBS, by applying on top of the tissue and incubate for 5 min at room temperature.

45 Remove the formaldehyde by pipetting it off and quench the tissue with 1mL 1.25M glycine for 5
min at room temperature.

46 Wash the slides with 1 mL of PBS for 1 minute and then dip the slide in a 50 mL falcon tube with DI water.

47 Dry the section with gentle air flow.

48 Take a full pre-scan image of the section at the desired optical resolution (Figure 4A).

Recommended 10X resolution.

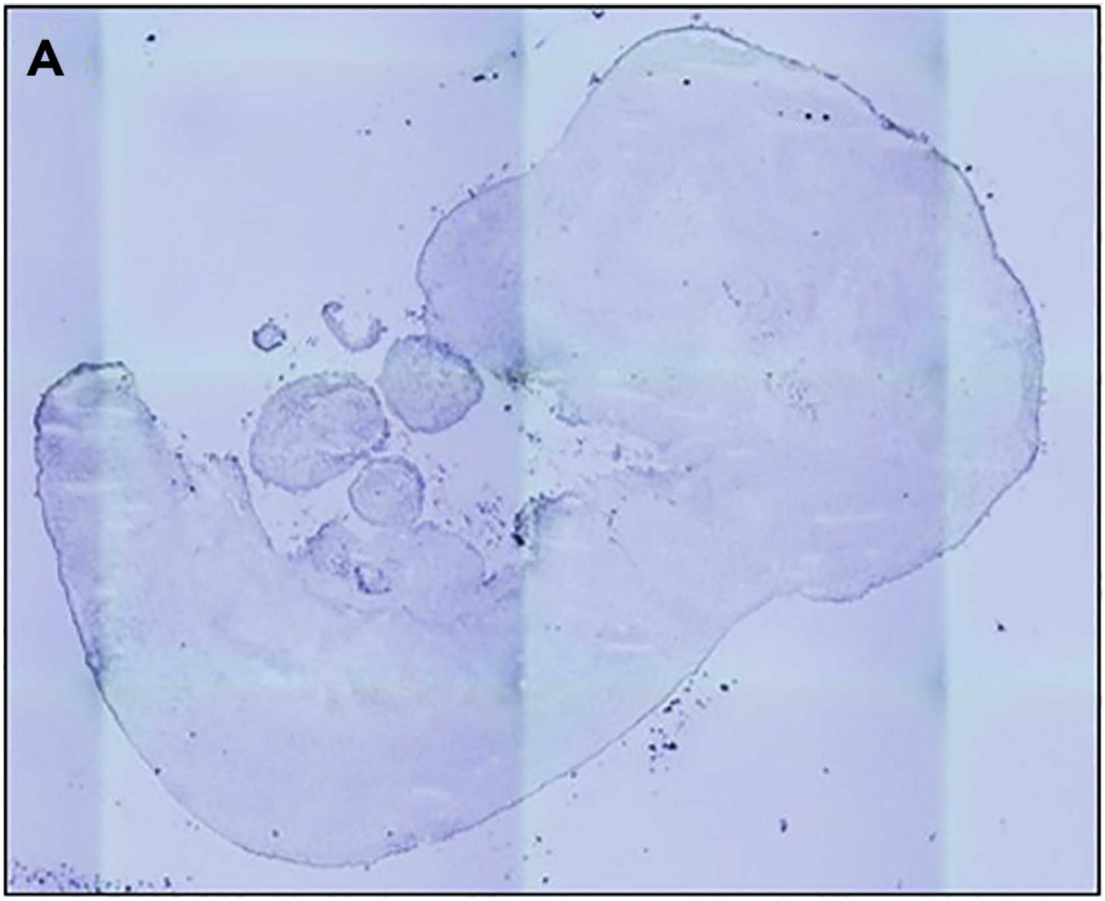


Figure 4 : Example scanning of the tissue slide.
(A) Initial full scan of the tissue section prior to DBiT-seq.

49 Attach the Antibody PDMS reservoir and 12mm diameter clamp to the tissue.

50 Permeabilize the tissue with 500uL 0.1 Xlysis buffer and incubate at room temperature for 15 min

Make sure to prepare 0.1x lysis buffer fresh each time. using the following reagents:
1X Lysis Buffer (10 mM Tris-HCl, pH 8.0; 10 mM NaCl; 3
mM MgCl2; 0.1% Tween-20; 0.1% NP-40; 0.01% Digitonin; 1% BSA)
Dilute down to 0.1X with lysis dilution buffer
(10 mM Tris-HCl, pH 8.0; 10 mM NaCl; 3 mM MgCl2; 1% BSA)

51 Wash the tissue with 500uL wash buffer for 5 min
make sure to prepare wash buffer fresh each time:

Wash Buffer (10 mM Tris-HCl pH 8.0; 10 mM NaCl; 3 mM MgCl2; 1% BSA; 0.1% Tween-20)

52 Prepare the transposition mix with the loaded Tn5 transposon and add 100uL to the tissue.

make sure to prepare the Tn5 mix fresh each time

	A	B	C	D
	Volume (200 u l/chip)	Volume (300 ul)	Volume (400 ul)	Reagent (50 ul/reaction)
	100 ul	150 ul	200 ul	2X TD Buffer (25 ul)
	66 ul	99 ul	132 ul	1X PBS (16.5 ul)
	2 ul	3 ul	4 ul	10% Tween-20 (final 0.1% v/v) (0.5 ul)
	2 ul	3 ul	4 ul	1% Digitonin (final 0.01% v/v) (0.5 ul)
	10 ul	15 ul	20 ul	Transposome (2.5 ul)
	20 ul	30 ul	40 ul	Nuclease-free H2O (5 ul)

53 Incubate the tissue at 37 ºC for 30 min

54 Stop the tagmentation reaction by adding 500 µL of 40 mM EDTA onto the tissue and incubating at room temperature for 5 min.

55 Dip in a 50 mL falcon tube with DI water and gently dry with air flow.

DBiT-seq assembly and Barcoding

56 Refer to https://www.protocols.io/view/spatial-multi-omics-sequencing-for-fixed-tissue-vi-3byl4j2kolo5/v1

Lysis

57 Take a small PDMS solution reservoir and place it on the tissue slide so that the reservoir opening is directly over the barcoded ROI.

58 Load the slide into a lysis clamping device and tighten screws as much as possible by hand.

59 Image the lysis chip on the ROI using a 10x objective lens.

60 Prepare the reverse crosslinking lysis solution.

Reagent	Volume	Stock Concentration	Final Concentration
Tris-HCl (pH 8.0)	7.5 µL	1M	50 mM
EDTA	0.3 µL	0.5M	1 mM
SDS	15 µL	10%	1%
NaCl	6 µL	5M	200 mM
proteinase K	3 µL	20mg/mL	0.4 mg/mL
dH2O	118.2 µL	-	-

61 Add approximately 50- 100µL of the reverse crosslinking lysis solution into the lysis chip. CRITICAL STEP Make sure to record the volume added of the lysis solution for the remaining steps.

62 Cover the ROI hole using parafilm and load the slide into a humidifying chamber.

63 Incubate tissue the slide with PDMS reservoir and Lysis Solution at 58 ºC for 2 hours to reverse formaldehyde crosslinks.

64 After incubation, collect the lysate in a 200µL PCR tube (or 1.5mL tube) and seal the tube with parafilm.

65 Incubate the lysate at 65 ºC with rotation overnight.

Purification and Library Generation

66 If necessary, transfer the lysate to a 1.5mL microcentrifuge tube.

67 During this transfer, carefully use a P200 pipette to measure the volume of the lysate, for an accurate PCR product: DNA fragment ratio in the Zymo purification.

68 Purify the lysate with the Zymo DNA Clean & Concentrator-5 according to the kit protocol:
a. Follow the 5:1 DNA Binding Buffer: DNA Sample
Use a pipette to accurately determine the initial volume of the PCR Product
b.For the final elution, elute with 100 uL of DNA Elution buffer

PCR

2h

69 Prepare the PCR solution:

a. 21uL purified DNA
b. 2.5uL 25uM Universal or barcoded i5 primer
c. 2.5uL 25uM Universal or barcoded i7 primer
(using different indices if needed)
d. 25uL 2X NEBNext Master Mix
Pipette up and down to mix once you have added everything.
Briefly vortex and spin down the tubes.
Place the tube in the NEW thermocycler and run the following protocol with a heated lid:
a. Lid should be heated at 105°C

b. Used default ramp rate

	Steps	Temperature	Time	Cycles
	Initial Denaturation	72˚C	5 min	1
	Denaturation	98˚C	30 sec	
	Denaturation	98˚C	10 sec	5
	Annealing	63˚C	30 sec	
	Extension	72˚C	1 min	

PCR Cycling Conditions

70 Determine Additional Cycles

1. Prepare preliminary qPCR solution :

- Pre-amplified library , 5uL
- Pre-amplified solution,0.5uL
- 25uM Universal or barcoded i5 primer, 0.5uL
- 25uM Universal or barcoded i7 primer (using different indices if needed), 5uL
- NEBNext Master Mix, 3.25uL
- Fuclease-free water, 0.75uL
- For a final concentration of 1X EvaGreen Dye, 0.75 uL

Pipette up and down to mix once you have added everything.
Briefly vortex and spin down the tubes.
Place the tube in the NEW thermocycler and run the following protocol with a heated lid:

- Lid should be heated at 105°C
- Use default ramp rate

Denaturation	98˚C	30 sec	
Denaturation	98˚C	10 sec	20 cylces
Annealing	63˚C	30 sec	
Extension	72˚C	1 min	

2. Use the qPCR data in Excel to determine the cycle where you reach 1/3 of the saturated signal in qPCR

- Graph the qPCR fluorescence values
- It's a good run if it plateaus in less than 10 cycles
- ((fluorescence value at cycle 20 - fluorescence value at cycle 1)/3) + fluorescence value at cycle 1 = 1/3 Saturated Signal Fluorescence Value

- Determine how many cycles it takes to get to this 1/3 Saturated Signal Fluorescence Value. If in the middle of two cycles, always take the lower.

Note: Sometimes, all the graphed values may be negative if the amount of DNA Is very high. This is fine, and just proceed to determine the number of cycles.

71 Repeat step 68 PCR protocol to amplify the remaining product with the cycles determined in step 69

Ampure Purification

40m

72 Perform a 1X SPRI purification by adding 45uL Ampure XP beads to the sample. Mix thoroughly by pipetting or vortexing.

73 Mix thoroughly by pipetting.

74 Keep the tubes on the magnet and add 200 µL of 80% ethanol.

75 Carefully remove and discard the supernatant.

76 Incubate the tubes at room temperature for at least 30 sec.

77 Carefully remove and discard the ethanol.

78 Repeat steps 70-75.

79 Remove the tubes from the magnet and resuspend the beads in 15-20 µL of PCR-grade water or elution buffer depending on downstream application.

80 Incubate the tubes at room temperature for at least 10 min to elute the cDNA off the beads.

81 Place the tubes back on the magnet and incubate until the liquid is fully clear.

82 Transfer the clear supernatant to a new tube.

83 Perform Bioanalyzer QC to obtain cDNA length profile and concentration.

Pause Point: Stop here for the second day. Store at 4˚C for 1-2 weeks or up to 6 months at -20˚C.

Sequencing

84 Send the samples for 100 million reads (30 Gb) and 150 bp paired end sequencing.

Data Processing

85 Transfer the two filtered sequences from Step 125 to Cell Ranger ATAC format (10x Genomics)

86 Align the filtered reads to the reference genome (e.g., mouse reference (mm10) or human reference (GRCh38) genome)

87 Adjust the reads for the Tn5 offset and generate an HTML report with per-base quality scores from which the overall sequencing quality of the library can be inferred. This step provides adjusted reads to correct the Tn5 enzyme's tendency to insert sequencing adapters from true chromatin accessibility sites at positions offset.

88 Perform peak calling and generate the number of Tn5 insertion events and fragments, generating a matrix with spatial barcodes. This step generates a BED-like fragment files for data visualization through Seurat packages.

89 Evaluate the molecular mechanism of binding and transposition by the Tn5 transposase dimer by generating fragment size distribution plots.

90 The Snakemake workflow management system (v5.28.0) pretreatment pipeline was created and used for preprocessing data, including trimming and alignment, and it is publicly available on GitHub at https://github.com/dyxmvp/Spatial_ATAC-seq.