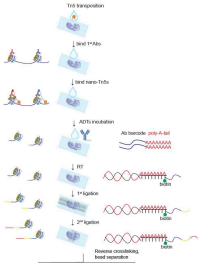
Oct 22, 2024

spatial-Mux-seq



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

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Keywords: Spatial multi-omics, nano-CUT&Tag, co-profiling of five modalities, microfluidic in situ, open chromatin at the same time, including multiple epigenomic modality, open chromatin, multiple epigenomic modality, whole transcriptome, histone modification, cellular level, generation sequencing, mux, tissue scale, sequencing

Abstract

Spatial-Mux-seq is a multi-modal spatial technology that allows simultaneous profiling of up to five different modalities, including multiple epigenomic modalities (any combination of two histone modifications and open chromatin at the same time), whole transcriptome, and a panel of proteins at tissue scale and cellular level in a spatially resolved manner. This was achieved by integrating microfluidic *in situ* barcoding and the nanobody-tethered transposition chemistry directly in tissue followed by high-throughput Next-Generation Sequencing.

Materials

Preparation of buffers:

DNA oligos used for PCR and preparation of sequencing library.

	RT-primer	/5Phos/CATCGGCGTACGACTNNNNNNNNNN/iBiodT/TTTTTTTTTTTTTTTVN
	template switch primer	AAGCAGTGGTATCAACGCAGAGTGAATrGrG+G
	Ligation linker 1	AGTCGTACGCCGATGCGAAACATCGGCCAC
	Ligation linker 2	CGAATGCTCTGGCCTCTCAAGCACGTGGAT
	PCR primer 1	CAAGCGTTGGCTTCTCGCATCT
	PCR primer 2	AAGCAGTGGTATCAACGCAGAGT
	primer 3 (cite-seq)	CCTTGGCACCCGAGAATT*C*C
	P5 oligo (cite-seq)	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTCAGAT

		GTGTATAAGAGACAGCCTT GGCACCCGAGAATTCC
	N501	AATGATACGGCGACCACC GAGATCTACACTAGATCGC TCGTCGGCAGCGTCAGAT GTGTATAAGAGACAG
	N701	CAAGCAGAAGACGGCATA CGAGATTCGCCTTAGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N702	CAAGCAGAAGACGGCATA CGAGATCTAGTACGGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N703	CAAGCAGAAGACGGCATA CGAGATTTCTGCCTGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N704	CAAGCAGAAGACGGCATA CGAGATGCTCAGGAGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N705	CAAGCAGAAGACGGCATA CGAGATAGGAGTCCGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N706	CAAGCAGAAGACGGCATA CGAGATCATGCCTAGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N707	CAAGCAGAAGACGGCATA

		CGAGATGTAGAGAGGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N709	CAAGCAGAAGACGGCATA CGAGATAGCGTAGCGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N710	CAAGCAGAAGACGGCATA CGAGATCAGCCTCGGTCT CGTGGGCTCGGAGATGTG TATAAGAGACAGCAAGCGT TGGCTTCTCGCATCT
	N711	CAAGCAGAAGACGGCATA CGAGATTGCCTCTTGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT
	N712	CAAGCAGAAGACGGCATA CGAGATTCCTCTACGTCTC GTGGGCTCGGAGATGTGTA TAAGAGACAGCAAGCGTTG GCTTCTCGCATCT

Supplementary Table 5.

Barcode A Sequence

	Barcode A-1	/5Phos/AGGCCAGAGCATT CGAACGTGATGTGGCCGA TGTTTCG
	Barcode A-2	/5Phos/AGGCCAGAGCATT CGAAACATCGGTGGCCGA TGTTTCG
	Barcode A-3	/5Phos/AGGCCAGAGCATT CGATGCCTAAGTGGCCGAT

		GTTTCG
	Barcode A-4	/5Phos/AGGCCAGAGCATTCGAGTGGTCAGTGGCCGATGTTTCG
	Barcode A-5	/5Phos/AGGCCAGAGCATTCGACCACTGTGTGGCCGATGTTTCG
	Barcode A-6	/5Phos/AGGCCAGAGCATTCGACATTGGCGTGGCCGATGTTTCG
	Barcode A-7	/5Phos/AGGCCAGAGCATTCGCAGATCTGGTGGCCGATGTTTCG
	Barcode A-8	/5Phos/AGGCCAGAGCATTCGCATCAAGTGTGGCCGATGTTTCG
	Barcode A-9	/5Phos/AGGCCAGAGCATTCGCGCTGATCGTGGCCGATGTTTCG
	Barcode A-10	/5Phos/AGGCCAGAGCATTCGACAAGCTAGTGGCCGATGTTTCG
	Barcode A-11	/5Phos/AGGCCAGAGCATTCGCTGTAGCCGTGGCCGATGTTTCG
	Barcode A-12	/5Phos/AGGCCAGAGCATTCGAGTACAAGGTGGCCGATGTTTCG
	Barcode A-13	/5Phos/AGGCCAGAGCATTCGAACAACCAGTGGCCGA

		TGTTTCG
	Barcode A-14	/5Phos/AGGCCAGAGCATTCGAACCGAGAGTGGCCGATGTTTCG
	Barcode A-15	/5Phos/AGGCCAGAGCATTCGAACGCTTAGTGGCCGATGTTTCG
	Barcode A-16	/5Phos/AGGCCAGAGCATTCGAAGACGGAGTGGCCGATGTTTCG
	Barcode A-17	/5Phos/AGGCCAGAGCATTCGAAGGTACAGTGGCCGATGTTTCG
	Barcode A-18	/5Phos/AGGCCAGAGCATTCGACACAGAAGTGGCCGATGTTTCG
	Barcode A-19	/5Phos/AGGCCAGAGCATTCGACAGCAGAGTGGCCGATGTTTCG
	Barcode A-20	/5Phos/AGGCCAGAGCATTCGACCTCCAAGTGGCCGATGTTTCG
	Barcode A-21	/5Phos/AGGCCAGAGCATTCGACGCTCGAGTGGCCGATGTTTCG
	Barcode A-22	/5Phos/AGGCCAGAGCATTCGACGTATCAGTGGCCGATGTTTCG
	Barcode A-23	/5Phos/AGGCCAGAGCATTCGACTATGCAGTGGCCGAT

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	Barcode A-24	/5Phos/AGGCCAGAGCATTCGAGAGTCAAGTGGCCGATGTTTCG
	Barcode A-25	/5Phos/AGGCCAGAGCATTCGAGATCGCAGTGGCCGATGTTTCG
	Barcode A-26	/5Phos/AGGCCAGAGCATTCGAGCAGGAAGTGGCCGATGTTTCG
	Barcode A-27	/5Phos/AGGCCAGAGCATTCGAGTCACTAGTGGCCGATGTTTCG
	Barcode A-28	/5Phos/AGGCCAGAGCATTCGATCCTGTAGTGGCCGATGTTTCG
	Barcode A-29	/5Phos/AGGCCAGAGCATTCGATTGAGGAGTGGCCGATGTTTCG
	Barcode A-30	/5Phos/AGGCCAGAGCATTCGCAACCACAGTGGCCGATGTTTCG
	Barcode A-31	/5Phos/AGGCCAGAGCATTCGGACTAGTAGTGGCCGATGTTTCG
	Barcode A-32	/5Phos/AGGCCAGAGCATTCGCAATGGAAGTGGCCGATGTTTCG
	Barcode A-33	/5Phos/AGGCCAGAGCATTCGCACTTCGAGTGGCCGA

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	Barcode A-34	/5Phos/AGGCCAGAGCATTCGCAGCGTTAGTGGCCGATGTTTCG
	Barcode A-35	/5Phos/AGGCCAGAGCATTCGCATACCAAGTGGCCGATGTTTCG
	Barcode A-36	/5Phos/AGGCCAGAGCATTCGCCAGTTCAGTGGCCGATGTTTCG
	Barcode A-37	/5Phos/AGGCCAGAGCATTCGCCGAAGTAGTGGCCGATGTTTCG
	Barcode A-38	/5Phos/AGGCCAGAGCATTCGCCGTGAGAGTGGCCGATGTTTCG
	Barcode A-39	/5Phos/AGGCCAGAGCATTCGCCTCCTGAGTGGCCGATGTTTCG
	Barcode A-40	/5Phos/AGGCCAGAGCATTCGCGAACTTAGTGGCCGATGTTTCG
	Barcode A-41	/5Phos/AGGCCAGAGCATTCGCGACTGGAGTGGCCGATGTTTCG
	Barcode A-42	/5Phos/AGGCCAGAGCATTCGCGCATACAGTGGCCGATGTTTCG
	Barcode A-43	/5Phos/AGGCCAGAGCATTCGCTCAATGAGTGGCCGA

		TGTTTCG
	Barcode A-44	/5Phos/AGGCCAGAGCATTCGCTGAGCCAGTGGCCGATGTTTCG
	Barcode A-45	/5Phos/AGGCCAGAGCATTCGCTGGCATAGTGGCCGATGTTTCG
	Barcode A-46	/5Phos/AGGCCAGAGCATTCGGAATCTGAGTGGCCGATGTTTCG
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	Barcode A-51	/5Phos/AGGCCAGAGCATTCGGCGAGTAAGTGGCCGATGTTTCG
	Barcode A-52	/5Phos/AGGCCAGAGCATTCGGCTAACGAGTGGCCGATGTTTCG
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	TGTTTCG
Barcode A-54	/5Phos/AGGCCAGAGCATTCGGGAGAACAGTGGCCGATGTTTCG
Barcode A-55	/5Phos/AGGCCAGAGCATTCGGGTGCGAAGTGGCCGATGTTTCG
Barcode A-56	/5Phos/AGGCCAGAGCATTCGGTACGCAAGTGGCCGATGTTTCG
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Barcode A-61	/5Phos/AGGCCAGAGCATTCGTATCAGCAGTGGCCGATGTTTCG
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Barcode A-63	/5Phos/AGGCCAGAGCATTCGTCTTCACAGTGGCCGAT

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	Barcode A-64	/5Phos/AGGCCAGAGCATTCGTGAAGAGAGTGGCCGATGTTTCG
	Barcode A-65	/5Phos/AGGCCAGAGCATTCGTGGAACAAGTGGCCGATGTTTCG
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	Barcode A-71	/5Phos/AGGCCAGAGCATTCGAAGGACACGTGGCCGATGTTTCG
	Barcode A-72	/5Phos/AGGCCAGAGCATTCGAATCCGTCGTGGCCGATGTTTCG
	Barcode A-73	/5Phos/AGGCCAGAGCATTCGAATGTTGCGTGGCCGA

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	Barcode A-74	/5Phos/AGGCCAGAGCATTCGACACGACCGTGGCCGATGTTTCG
	Barcode A-75	/5Phos/AGGCCAGAGCATTCGACAGATTCGTGGCCGATGTTTCG
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	Barcode A-77	/5Phos/AGGCCAGAGCATTCGAGCACCTCGTGGCCGATGTTTCG
	Barcode A-78	/5Phos/AGGCCAGAGCATTCGAGCCATGCGTGGCCGATGTTTCG
	Barcode A-79	/5Phos/AGGCCAGAGCATTCGAGGCTAACGTGGCCGATGTTTCG
	Barcode A-80	/5Phos/AGGCCAGAGCATTCGATAGCGACGTGGCCGATGTTTCG
	Barcode A-81	/5Phos/AGGCCAGAGCATTCGATCATTCCGTGGCCGATGTTTCG
	Barcode A-82	/5Phos/AGGCCAGAGCATTCGATTGGCTCGTGGCCGATGTTTCG
	Barcode A-83	/5Phos/AGGCCAGAGCATTCGCAAGGAGCGTGGCCGA

		TGTTTCG
	Barcode A-84	/5Phos/AGGCCAGAGCATTCGCACCTTACGTGGCCGATGTTTCG
	Barcode A-85	/5Phos/AGGCCAGAGCATTCGCCATCCTCGTGGCCGATGTTTCG
	Barcode A-86	/5Phos/AGGCCAGAGCATTCGCCGACAACGTGGCCGATGTTTCG
	Barcode A-87	/5Phos/AGGCCAGAGCATTCGCCTAATCCGTGGCCGATGTTTCG
	Barcode A-88	/5Phos/AGGCCAGAGCATTCGCCTCTATCGTGGCCGATGTTTCG
	Barcode A-89	/5Phos/AGGCCAGAGCATTCGCGACACACGTGGCCGATGTTTCG
	Barcode A-90	/5Phos/AGGCCAGAGCATTCGCGGATTGCGTGGCCGATGTTTCG
	Barcode A-91	/5Phos/AGGCCAGAGCATTCGCTAAGGTCGTGGCCGATGTTTCG
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	Barcode A-98	/5Phos/AGGCCAGAGCATTCGGTGACAAGGTGGCCGATGTTTCG
	Barcode A-99	/5Phos/AGGCCAGAGCATTCGGAACCAGAGTGGCCGATGTTTCG
	Barcode A-100	/5Phos/AGGCCAGAGCATTCGTTGCTGGAGTGGCCGATGTTTCG

Supplementary Table 6.

Barcode B Sequence

	Barcode B-1	AAGCGTTGGCTTCTCGCATCTAACGTGATATCCACGTGCTTGAG
	Barcode B-2	CAAGCGTTGGCTTCTCGC

		ATCTAAACATCGATCCACG TGCTTGAG
	Barcode B-3	CAAGCGTTGGCTTCTCGC ATCTATGCCTAAATCCACG TGCTTGAG
	Barcode B-4	CAAGCGTTGGCTTCTCGC ATCTAGTGGTCAATCCACG TGCTTGAG
	Barcode B-5	CAAGCGTTGGCTTCTCGC ATCTACCACTGTATCCACG TGCTTGAG
	Barcode B-6	CAAGCGTTGGCTTCTCGC ATCTACATTGGCATCCACG TGCTTGAG
	Barcode B-7	CAAGCGTTGGCTTCTCGC ATCTCAGATCTGATCCACG TGCTTGAG
	Barcode B-8	CAAGCGTTGGCTTCTCGC ATCTCATCAAGTATCCACG TGCTTGAG
	Barcode B-9	CAAGCGTTGGCTTCTCGC ATCTCGCTGATCATCCACG TGCTTGAG
	Barcode B-10	CAAGCGTTGGCTTCTCGC ATCTACAAGCTAATCCACG TGCTTGAG
	Barcode B-11	CAAGCGTTGGCTTCTCGC ATCTCTGTAGCCATCCACG TGCTTGAG

	Barcode B-12	CAAGCGTTGGCTTCTCGC ATCTAGTACAAGATCCACG TGCTTGAG
	Barcode B-13	CAAGCGTTGGCTTCTCGC ATCTAACAACCAATCCACG TGCTTGAG
	Barcode B-14	CAAGCGTTGGCTTCTCGC ATCTAACCGAGAATCCACG TGCTTGAG
	Barcode B-15	CAAGCGTTGGCTTCTCGC ATCTAACGCTTAATCCACG TGCTTGAG
	Barcode B-16	CAAGCGTTGGCTTCTCGC ATCTAAGACGGAATCCACG TGCTTGAG
	Barcode B-17	CAAGCGTTGGCTTCTCGC ATCTAAGGTACAATCCACG TGCTTGAG
	Barcode B-18	CAAGCGTTGGCTTCTCGC ATCTACACAGAAATCCACG TGCTTGAG
	Barcode B-19	CAAGCGTTGGCTTCTCGC ATCTACAGCAGAATCCACG TGCTTGAG
	Barcode B-20	CAAGCGTTGGCTTCTCGC ATCTACCTCCAAATCCACG TGCTTGAG
	Barcode B-21	CAAGCGTTGGCTTCTCGC ATCTACGCTCGAATCCACG TGCTTGAG

	Barcode B-22	CAAGCGTTGGCTTCTCGC ATCTACGTATCAATCCACG TGCTTGAG
	Barcode B-23	CAAGCGTTGGCTTCTCGC ATCTACTATGCAATCCACG TGCTTGAG
	Barcode B-24	CAAGCGTTGGCTTCTCGC ATCTAGAGTCAAATCCACG TGCTTGAG
	Barcode B-25	CAAGCGTTGGCTTCTCGC ATCTAGATCGCAATCCACG TGCTTGAG
	Barcode B-26	CAAGCGTTGGCTTCTCGC ATCTAGCAGGAAATCCACG TGCTTGAG
	Barcode B-27	CAAGCGTTGGCTTCTCGC ATCTAGTCACTAATCCACG TGCTTGAG
	Barcode B-28	CAAGCGTTGGCTTCTCGC ATCTATCCTGTAATCCACG TGCTTGAG
	Barcode B-29	CAAGCGTTGGCTTCTCGC ATCTATTGAGGAATCCACG TGCTTGAG
	Barcode B-30	CAAGCGTTGGCTTCTCGC ATCTCAACCACAATCCACG TGCTTGAG
	Barcode B-31	CAAGCGTTGGCTTCTCGC ATCTGACTAGTAATCCACG TGCTTGAG

	Barcode B-32	CAAGCGTTGGCTTCTCGC ATCTCAATGGAAATCCACG TGCTTGAG
	Barcode B-33	CAAGCGTTGGCTTCTCGC ATCTCACTTCGAATCCACG TGCTTGAG
	Barcode B-34	CAAGCGTTGGCTTCTCGC ATCTCAGCGTTAATCCACG TGCTTGAG
	Barcode B-35	CAAGCGTTGGCTTCTCGC ATCTCATACCAAATCCACG TGCTTGAG
	Barcode B-36	CAAGCGTTGGCTTCTCGC ATCTCCAGTTCAATCCACG TGCTTGAG
	Barcode B-37	CAAGCGTTGGCTTCTCGC ATCTCCGAAGTAATCCACG TGCTTGAG
	Barcode B-38	CAAGCGTTGGCTTCTCGC ATCTCCGTGAGAATCCACG TGCTTGAG
	Barcode B-39	CAAGCGTTGGCTTCTCGC ATCTCCTCCTGAATCCACG TGCTTGAG
	Barcode B-40	CAAGCGTTGGCTTCTCGC ATCTCGAACTTAATCCACG TGCTTGAG
	Barcode B-41	CAAGCGTTGGCTTCTCGC ATCTCGACTGGAATCCACG TGCTTGAG

	Barcode B-42	CAAGCGTTGGCTTCTCGC ATCTCGCATACAATCCACG TGCTTGAG
	Barcode B-43	CAAGCGTTGGCTTCTCGC ATCTCTCAATGAATCCACG TGCTTGAG
	Barcode B-44	CAAGCGTTGGCTTCTCGC ATCTCTGAGCCAATCCACG TGCTTGAG
	Barcode B-45	CAAGCGTTGGCTTCTCGC ATCTCTGGCATAATCCACG TGCTTGAG
	Barcode B-46	CAAGCGTTGGCTTCTCGC ATCTGAATCTGAATCCACG TGCTTGAG
	Barcode B-47	CAAGCGTTGGCTTCTCGC ATCTCAAGACTAATCCACG TGCTTGAG
	Barcode B-48	CAAGCGTTGGCTTCTCGC ATCTGAGCTGAAATCCACG TGCTTGAG
	Barcode B-49	CAAGCGTTGGCTTCTCGC ATCTGATAGACAATCCACG TGCTTGAG
	Barcode B-50	CAAGCGTTGGCTTCTCGC ATCTGCCACATAATCCACG TGCTTGAG
	Barcode B-51	CAAGCGTTGGCTTCTCGC ATCTGCGAGTAAATCCACG TGCTTGAG

	Barcode B-52	CAAGCGTTGGCTTCTCGC ATCTGCTAACGAATCCACG TGCTTGAG
	Barcode B-53	CAAGCGTTGGCTTCTCGC ATCTGCTCGGTAATCCACG TGCTTGAG
	Barcode B-54	CAAGCGTTGGCTTCTCGC ATCTGGAGAACAATCCACG TGCTTGAG
	Barcode B-55	CAAGCGTTGGCTTCTCGC ATCTGGTGCGAAATCCACG TGCTTGAG
	Barcode B-56	CAAGCGTTGGCTTCTCGC ATCTGTACGCAAATCCACG TGCTTGAG
	Barcode B-57	CAAGCGTTGGCTTCTCGC ATCTGTCGTAGAATCCACG TGCTTGAG
	Barcode B-58	CAAGCGTTGGCTTCTCGC ATCTGTCTGTCAATCCACG TGCTTGAG
	Barcode B-59	CAAGCGTTGGCTTCTCGC ATCTGTGTTCTAATCCACG TGCTTGAG
	Barcode B-60	CAAGCGTTGGCTTCTCGC ATCTTAGGATGAATCCACG TGCTTGAG
	Barcode B-61	CAAGCGTTGGCTTCTCGC ATCTTATCAGCAATCCACG TGCTTGAG

	Barcode B-62	CAAGCGTTGGCTTCTCGC ATCTTCCGTCTAATCCACG TGCTTGAG
	Barcode B-63	CAAGCGTTGGCTTCTCGC ATCTTCTTCACAATCCACG TGCTTGAG
	Barcode B-64	CAAGCGTTGGCTTCTCGC ATCTTGAAGAGAATCCACG TGCTTGAG
	Barcode B-65	CAAGCGTTGGCTTCTCGC ATCTTGGAACAAATCCACG TGCTTGAG
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	Barcode B-69	CAAGCGTTGGCTTCTCGC ATCTAACTCACCATCCACG TGCTTGAG
	Barcode B-70	CAAGCGTTGGCTTCTCGC ATCTAAGAGATCATCCACG TGCTTGAG
	Barcode B-71	CAAGCGTTGGCTTCTCGC ATCTAAGGACACATCCACG TGCTTGAG

	Barcode B-72	CAAGCGTTGGCTTCTCGC ATCTAATCCGTCATCCACG TGCTTGAG
	Barcode B-73	CAAGCGTTGGCTTCTCGC ATCTAATGTTGCATCCACG TGCTTGAG
	Barcode B-74	CAAGCGTTGGCTTCTCGC ATCTACACGACCATCCACG TGCTTGAG
	Barcode B-75	CAAGCGTTGGCTTCTCGC ATCTACAGATTCATCCACG TGCTTGAG
	Barcode B-76	CAAGCGTTGGCTTCTCGC ATCTAGATGTACATCCACG TGCTTGAG
	Barcode B-77	CAAGCGTTGGCTTCTCGC ATCTAGCACCTCATCCACG TGCTTGAG
	Barcode B-78	CAAGCGTTGGCTTCTCGC ATCTAGCCATGCATCCACG TGCTTGAG
	Barcode B-79	CAAGCGTTGGCTTCTCGC ATCTAGGCTAACATCCACG TGCTTGAG
	Barcode B-80	CAAGCGTTGGCTTCTCGC ATCTATAGCGACATCCACG TGCTTGAG
	Barcode B-81	CAAGCGTTGGCTTCTCGC ATCTATCATTCCATCCACG TGCTTGAG

	Barcode B-82	CAAGCGTTGGCTTCTCGC ATCTATTGGCTCATCCACG TGCTTGAG
	Barcode B-83	CAAGCGTTGGCTTCTCGC ATCTCAAGGAGCATCCACG TGCTTGAG
	Barcode B-84	CAAGCGTTGGCTTCTCGC ATCTCACCTTACATCCACG TGCTTGAG
	Barcode B-85	CAAGCGTTGGCTTCTCGC ATCTCCATCCTCATCCACG TGCTTGAG
	Barcode B-86	CAAGCGTTGGCTTCTCGC ATCTCCGACAACATCCACG TGCTTGAG
	Barcode B-87	CAAGCGTTGGCTTCTCGC ATCTCCTAATCCATCCACG TGCTTGAG
	Barcode B-88	CAAGCGTTGGCTTCTCGC ATCTCCTCTATCATCCACG TGCTTGAG
	Barcode B-89	CAAGCGTTGGCTTCTCGC ATCTCGACACACATCCACG TGCTTGAG
	Barcode B-90	CAAGCGTTGGCTTCTCGC ATCTCGGATTGCATCCACG TGCTTGAG
	Barcode B-91	CAAGCGTTGGCTTCTCGC ATCTCTAAGGTCATCCACG TGCTTGAG

	Barcode B-92	CAAGCGTTGGCTTCTCGC ATCTGAACAGGCATCCACG TGCTTGAG
	Barcode B-93	CAAGCGTTGGCTTCTCGC ATCTGACAGTGCATCCACG TGCTTGAG
	Barcode B-94	CAAGCGTTGGCTTCTCGC ATCTGAGTTAGCATCCACG TGCTTGAG
	Barcode B-95	CAAGCGTTGGCTTCTCGC ATCTGATGAATCATCCACG TGCTTGAG
	Barcode B-96	CAAGCGTTGGCTTCTCGC ATCTGCCAAGACATCCACG TGCTTGAG
	Barcode B-97	CAAGCGTTGGCTTCTCGC ATCTCGGAAGAAATCCACG TGCTTGAG
	Barcode B-98	CAAGCGTTGGCTTCTCGC ATCTGTGACAAGATCCACG TGCTTGAG
	Barcode B-99	CAAGCGTTGGCTTCTCGC ATCTGAACCAGAATCCACG TGCTTGAG
	Barcode B-100	CAAGCGTTGGCTTCTCGC ATCTTTGCTGGAATCCACG TGCTTGAG

Supplementary Table 7.
Chemicals and reagents

	A	B	C
	Name	**Catalog number**	**Vender**
	Formaldehyde solution	PI28906	Thermo Fisher Scientific
	HEPES pH 7.5	BBH-75-250	Boston BioProducts
	Glycine	50046	Sigma-Aldrich
	NaCl	AM9760G	Thermo Fisher Scientific
	Digitonin	G9441	Promega
	MgCl2	AM9530G	Thermo Fisher Scientific
	Spermidine	S0266	Sigma-Aldrich
	EDTA-free Protease Inhibitor Cocktail	11873580001	Millipore Sigma
	NP40	11332473001	Sigma-Aldrich
	EDTA Solution pH 8.0	AB00502	AmericanBio
	Bovine Serum Albumin (BSA)	A8806	Sigma-Aldrich
	Anti-H3K27me3 antibody	Ab6002	Abcam
	Anti-H3K27ac antibody	8173	Cell Signaling Technology
	Anti-H3K4me3 antibody	9751	Cell Signaling Technology

	A	B	C
	TotalSeq‰-A Mouse Universal Cocktail	199901	Biolegend
	Anti mouse CD4	A0001	Biolegend
	Anti mouse CD3	A0182	Biolegend
	Anti mouse CD34	A0857	Biolegend
	Anti mouse CD140a	A0573	Biolegend
	Anti mouse CD133	A1037	Biolegend
	Anti mouse CD90.1	A0380	Biolegend
	Anti mouse CD90.2	A0075	Biolegend
	Anti mouse B220	A0103	Biolegend
	TruStain FcX‰ PLUS (anti-mouse CD16/32) antibody	156604	Biolegend
	Cell Staining Buffer	420201	Biolegend
	Fab Fragment Goat Anti-Mouse IgG	115-007-003	Jackson ImmunoResearch
	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

	A	B	C
	Proteinase K	EO0491	Thermo Fisher Scientific
	SPRI 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
	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
	Dynabeads‰ MyOne‰ Streptavidin C1	65001	Thermo Fisher Scientific
	RNase Inhibitor	Y9240L	Enzymatics
	Kapa Hotstart HiFi ReadyMix	KK2601	Kapa Biosystems

	A	B	C
	Nextera XT DNA Library Preparation Kit	FC-131-1024	Illumina

Safety warnings

Digitonin is toxic and care should be taken especially when weighing out the powder. Use full PPE including a mask, lab coat and gloves while handling any amount of digitonin.

Cryosectioning Procedure

1 Specimens should be handled with RNAse Away. 20m

> Note
>
> This protocol is to be used with tissue preserved in OCT and stored in -80 °C .

- Cool Cryostat to -20 °C . Clean the work surfaces with RNAse away and change a new cutting blade.
- Place a clean slide box inside the cryostat chamber to store slides.
- Clean the tissue holder with RNAse away and put it in the cryostat chamber . Adhere the OCT specimen to a tissue holder and allow it to equilibrate for 00:20:00 to reach the chamber temperature and strengthen the adhesion between the OCT block and the holder.
- Place the specimen in the cryostat with the tissue holder and cut at Thikness 8-10 µm.
- Store completed slides at -80 °C .

Tn5 loading 10m

2 **Annealing adaptor sequences:** 1h 35m

Tn5MErev:
5′-/Phos/CTGTCTCTTATACACATCT-3′
Tn5ME-A:
5′-/Phos/CATCGGCGTACGACTAGATGTGTATAAGAGACAG-3′
Tn5ME-B1:
5′-/Phos/CATCGGCGTACGACT**TAGCCT**AGATGTGTATAAGAGACAG-3′
Tn5ME-B2:
5′-/Phos/CATCGGCGTACGACT**ATAGAG**AGATGTGTATAAGAGACAG-3′
Tn5ME-B3:
5′-/Phos/CATCGGCGTACGACT**CCTATC**AGATGTGTATAAGAGACAG-3′

- Resuspend the oligonucleotides (Tn5MErev, Tn5ME-A, Tn5ME-B) in water to a final concentration of 100 µM each.

- Mix equimolar amounts of Tn5MErev/Tn5ME-A and Tn5MErev/Tn5ME-B in separate 200 µl PCR tubes.

  e.g:
  tube1: 10 µL Tn5ME-A + 10 µL Tn5MErev
  tube2: 10 µL Tn5ME-B + 10 µL Tn5MErev

- Denature in the thermocycler for 00:05:00 at 95 °C, and cool down slowly on the thermocycler by ramping down to 12 °C by 0.1 °C per second
  Pause point: Store the annealed oligos at -20 °C

- Mix annealing mix.

The final Tn5 concentration is 2 µM of Tn5 dimer.

Use unique barcodes for specific nano-Tn5 or WT-Tn5.

2x Tn5 loading buffer

2x Tn5 loading buffer		Can be stored at 4C without DTT *		
final conc	stock		stock	for 10ml
100 mM	HEPES pH 7.2		250 mM	4 ml
200 mM	NaCl		5M	400 ul
0.2 mM	EDTA		0.5M	4ul
0.2 %	Triton-X		10%	200 ul
20%	Glycerol		100%	2ml
2 mM	DTT		200 mM	*
	water			3.4 ml

Mouse Nano-Tn5 (MeA/B1/R loaded):

4 µL Annealed, barcoded MeA/Me-Rev oligos Concentration50 micromolar (µM)

4 µL Annealed, barcoded MeB1/Me-Rev oligos Concentration50 micromolar (µM)

42 µL Glycerol

6 µL Nano-Tn5 (mouse, 5mg/ml, MW = 73941 g/mol; Concentration67.6 micromolar (µM))

44 µL 2x Nano-Tn5 loading buffer

Rabbit Nano-Tn5 (MeA/B2/R loaded):

4 µL Annealed, barcoded MeA/Me-Rev oligos Concentration50 micromolar (µM)

4 µL Annealed, barcoded MeB2/Me-Rev oligos Concentration50 micromolar (µM)

42 µL Glycerol

4.4 µL Nano-Tn5 (rabbit, 6.8mg/ml, MW = 73013 g/mol Concentration93.1 micromolar (µM))

45.6 µL 2x Nano-Tn5 loading buffer

For nano-Tn5, incubate for 01:00:00 at 23 °C .

WT Tn5-MeA with barcode (MeA/B3/R for ATAC-seq)

5 µL Annealed, barcoded MeA/Me-Rev oligos Concentration50 micromolar (µM)

5 µL Annealed, barcoded MeB3/Me-Rev oligos Concentration50 micromolar (µM)

10 µL Tn5 (Order from Diagenode)

For WT-Tn5, incubate for 00:30:00 at 23 °C .

Then mix with 10 µL Glycerol.

Note

Store the loaded nano-Tn5 or WT-Tn5 at Temperature-20 °C.

Fixation

3 Frozen tissue slides were first thawed for 00:01:00 at 37 °C . 1m

4 Tissue was fixed with 1 mL [M] 0.2 % volume formaldehyde in PBS (with 0.05 U μl^{-1} RNase Inhibitor) at Room temperature for 00:05:00 . 5m

5 Discard reagent and quench with 1 mL [M] 1.25 Molarity (M) glycine (with 0.05 U μl^{-1} RNase Inhibitor) for 00:05:00 . 5m

6 Discard reagent and wash slide with 1x PBS (with 0.05 U μl^{-1} RNase Inhibitor) for 00:01:00 . 1m

7 Discard reagent and rinse slide sequentially in RNase free water. Wipe away excess liquid.

8 Allow slide to air dry and image the slide on a Keyence BZ-X810 scanning microscope at 10x or 20x resolution.

ATAC-seq 10m

9 **Buffer preparation for ATAC-seq**

Wash Buffer I

Reagent	1 ml	2 ml	Stock Concentration	Final Concentration
Tris-HCl (pH 7.4)	10 µl	20 µl	1 M	10 mM
NaCl	2 µl	4 µl	5 M	10 mM
MgCl2	3 µl	6 µl	1 M	3 mM
BSA	200 µl	400 µl	5%	1%
Tween-20	10 µl	20 µl	10%	0.1%
Nuclease-free Water	775µl	2 x775 µl	-	-

1X Lysis Buffer

Reagent	1 ml	2 ml	Stock Concentration	Final Concentration
Tris-HCl (pH 7.4)	10 µl	20 µl	1 M	10 mM
NaCl	2 µl	4 µl	5 M	10 mM
MgCl2	3 µl	6 µl	1 M	3 mM
Tween-20	10 µl	20 µl	10%	0.1%
NP-40	10 µl	20 µl	10%	0.1%
Digitonin	10 µl	20 µl	1%	0.01%
BSA	200 µl	400 µl	5%	1%
Nuclease-free Water	755µl	2 x755 µl	-	-

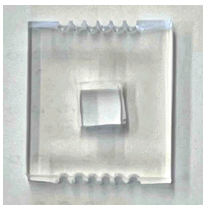
Lysis Dilution Buffer

Reagent	1ml	2 ml	Stock Concentration	Final Concentration
Tris-HCl (pH 7.4)	10 µl	20 µl	1 M	10 mM
NaCl	2 µl	4 µl	5 M	10 mM
MgCl2	3 µl	6 µl	1 M	3 mM
BSA	200 µl	400 µl	5%	1%
Nuclease-free Water	785µl	2 x785 µl	-	-

0.1X Lysis Buffer

Reagent	2 ml	Stock Concentration	Final Concentration
1X Lysis Buffer	200 µl	1X	0.1X
Lysis Dilution Buffer	2 ml – 200 µl	-	-

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



Note

The PDMS reservoir should cover the entire tissue region of interest on the slide, but small enough to save reagents.

11 Permeabilize the tissue with 200 µL 0.1X lysis buffer and incubate at Room temperature for 00:15:00 . 15m

12 Discard reagent and Wash the tissue with 200 µL wash buffer I at Room temperature for 00:05:00 . 5m

13 Discard reagent and quickly wash the tissue with 200 µL wash buffer.

14 Prepare transposition mix:

50 µL	2X TD Buffer (see materials section)
33 µL	1X PBS
1 µL	10% Tween-20 (final 0.1% v/v)

1 µL	1% Digitonin (final 0.01% v/v)
5 µL	Tn5 Transposase (WT Tn5)
10 µL	nuclease-free H_2O

15 Discard reagent and add 100 µL transposition mix.

16 Incubate for 00:30:00 at 37 °C. 30m

17 Discard reagent and stop the tagmentation by adding 200 µL of [M] 40 millimolar (mM) EDTA at Room temperature for 00:05:00. 5m

Nano-CUT&Tag

18 **Buffer preparation for Nano-CUT&Tag**

2 M spermidine:
~Prepare fresh each day

Reagent	Final concentration	Final 2 M volume (50 µL)
dH_2O	N/A	34.3 µL
spermidine		15.7 µL

Wash bufferII:
mix 1 ml 1 M HEPES pH 7.5, 1.5 ml 5 M NaCl, 12.5 µL 2 M spermidine, bring the final volume to 50 ml with dH_2O, and add 1 Roche Complete Protease Inhibitor EDTA-Free tablet.
Alternative: dissolve 1 tablet inhibitor into 2 ml dH_2O.
~Store @ 4°C for up to 1 week.

reagent	Final concentration	Volume for 50 ml	Volume for 10 ml	Volume for 5 ml	Volume for 2 ml	Volume for 1 ml
1 M HEPES pH 7.5	20 mM	1 mL	0.2 ml	100 µL	40 µL	20 µL
5 M NaCl	150 mM	1.5 mL	0.3 ml	150 µL	60 µL	30 µL
2 M spermidine	0.5 mM	12.5 µL	2.5 µL	1.25 µL	0.5 µL	0.25 µL
dH_2O		45.4875 ml	9.0974 ml	4.54875 ml	1.81948 ml	910 µL
Protease inhibitor		2 ml	0.4 ml	200 µL	80 µL	40 µL

NP40 Digitonin wash buffer (permeabilize):
0.01% NP40, 0.01% Digitonin in **wash buffer.**
~prepare fresh each day and store @4°C.

Reagent	Final concentration	Volume for 2 ml	Volume for 1.5 ml	Volume for 1 ml	Volume for 0.5 ml
10% NP40	0.01%	2 µL	1.5 µL	1 µL	0.5 µL
1% Digitonin	0.01%	20 µL	15 µL	10 µL	5 µL
Wash buffer		989 x 2 µL	741.75 x2 µL	989 µL	494.5 µL

Digitonin is supplied at 2% in DMSO. Dilute 1:1 with dH2O to make a 1% stock solution. Avoid more than five freeze thaw cycles. Can be kept at -20°C up to 6 Months.

Antibody buffer:

antibody buffer mixes 8 µL 0.5 M EDTA and 6.7 µL 30% BSA with 2 ml NP40-Digitonin wash buffer and chill on ice. (Mix 4 µL 0.5 M EDTA and 10 µL 1% BSA with 1 ml NP40-DIGITONIN wash buffer and chill on ice) ~prepare fresh each day and store @4°C。

Reagent	Final concentration	Volume for 1 ml	Volume for 0.5 ml	Volume for 0.25 ml
0.5 M EDTA	2 mM	4 µL	2 µL	1 µL
30% BSA	0.001%	3.35 µL	1.675 µL	0.8375 µL
NP40-Digitonin wash buffer	N/A	992.65 µL	496.325 µL	248.1625 µL

300-Wash buffer:

mix 1 ml 1 M HEPES pH 7.5, 3 ml 5 M NaCl, 12.5 µL 2 M spermidine, bring the final volume to 50 ml with dH2O, and add 1 Roche Complete Protease Inhibitor EDTA-Free tablet.
Alternative: dissolve 1 tablet inhibitor into 2 ml dH_2O.
~Store @ 4°C for up to 1 week.

reagent	Final concentration	Volume for 50 ml	Volume for 10 ml	Volume for 2 ml	Volume for 1 ml
1 M HEPES pH 7.5	20 mM	1 mL	0.2 ml	40 µL	20 µL
5 M NaCl	300 mM	3 mL	0.6 ml	120 µL	60 µL
2 M spermidine	0.5 mM	12.5 µL	2.5 µL	0.5 µL	0.25 µL
dH_2O		43.9875 ml	8.7974 ml	880 x 2 µL	880 µL
Protease inhibitor		2 ml	0.4 ml	80 µL	40 µL

tagmentation solution:

Mix 1ml 300-wash buffer and 10 µL 1 M $MgCl_2$ (final 10 mM)
~Store @4°C for up to 1 week

Reagent	Final concentration	Volume for 14 ml	Volume for 12 ml	Volume for 6 ml	Volume for 1 ml
1 M $MgCl_2$	10 mM	140 µL	120 µL	60 µL	10 µL
300-wash buffer		13.86 ml	11.88 µL	5.94 ml	990 µL

19 Discard reagent and Wash the tissue with 200 µL wash buffer II at Room temperature for 00:05:00 . 5m

20 Discard reagent and Wash the tissue with 200 µL NP40-Digitonin wash buffer at Room temperature for 00:05:00 . 5m

Primary antibody binding

21 Starting concentrations (can be further optimised, depending on the antibody)

1:50 primary antibody in antibody-binding buffer.

Final volume 100 µL per sample.

22 Discard reagent and add 100 µL primary antibody mix.

23 Incubate at 4 °C Overnight 20m

> **Note**
>
> Make sure the liquid can cover the tissue region of interest.

Nano-Tn5 binding

24 Discard reagent and Wash the tissue with 200 µL 300-wash buffer at Room temperature for 00:05:00 . 5m

25 Starting concentrations of nano-Tn5s mix

1:100 nano-Tn5s in 300-wash buffer.
Final volume 100 µL per sample.

26 Discard reagent and add 100 µL nano-Tn5s mix at Room temperature for 01:00:00 1h

27 Discard reagent and Wash the tissue with 200 µL 300-wash buffer at Room temperature for 00:05:00 . 5m

28 Discard reagent and add 200 µL Tagmentation buffer.

29 Incubate for 01:00:00 at 37 °C . 1h

30 Discard reagent and stop the tagmentation by adding 200 µL of [M] 40 millimolar (mM) EDTA at Room temperature for 00:05:00 . 5m

Surface Protein Antibody staining

31 Discard reagent and add 200 µL of cell surface staining buffer at Room temperature for 00:02:00 . 2m

32 Discard reagent and add 200 µL of 1:20 TruStain FcX™ in Cell Staining Buffer at 4 °C for 00:15:00 . 15m

33 Discard reagent and add 200 µL of cell surface staining buffer at Room temperature for 00:01:00 . 1m

34 Discard reagent and add 200 µL of oligonucleotide-labeled antibody-derived tags (1:100) in cell surface staining buffer.

35 Incubate for 00:30:00 at 4 °C . 30m

36 Discard reagent and add 200 µL of cell surface staining buffer at Room temperature for 00:01:00 . 1m

37 Discard reagent and add 200 µL of Fab Fragment Goat Anti-Mouse IgG (1:25) in cell surface staining buffer.

38 Incubate for 00:15:00 at 4 °C . 15m

In situ reverse transcription

39 Dsicard reagent and tissue was fixed with 200 µL [M] 2 % volume formaldehyde in PBS (with 0.05 U μl^{-1} RNase Inhibitor) at Room temperature for 00:10:00 . 10m

40 Discard reagent and quench with 1 mL [M] 1.25 Molarity (M) glycine (with 0.05 U μl^{-1} RNase Inhibitor) for 00:05:00 . 5m

41 Discard reagent and add 200 µL of 0.5x PBS at Room temperature for 00:05:00 . 5m

42 Prepare the RT mixture

For one sample:

12.5 µL	5X RT Buffer
0.8 µL	RNase inhibitor
3.1 µL	dNTPs
6.1 µL	Maxima H Minus Reverse Transcriptase
20.3 µL	0.5X PBS
10 µL	RT primer
9.6 µL	ddH_2O

43 Discard reagent and add 62.5 µL RT mixture.

44 Incubate at Room temperature 00:30:00 . 30m

45 Incubate at 42 °C 01:30:00 . 1h 30m

46 Discard reagent and add 200 µL of 1X NEBBuffer 3.1 at Room temperature for 00:05:00 . 5m

47 Discard reagent, remove the clamp, and dip the tissue slide into ddH_2O.

48 Air dry the slide.

In-tissue ligation with barcode A

49 **Prepare buffer:** 5m

2X annealing buffer:

[M] 2 millimolar (mM)	EDTA, pH 8.0
[M] 20 millimolar (mM)	Tris-HCl, pH 7.5
[M] 100 millimolar (mM)	NaCl

Prepare annealed barcode An

25 µL		2X annealing buffer
12.5 µL	[M] 100 micromolar (µM)	barcode An
12.5 µL	[M] 100 micromolar (µM)	ligation linker 1

Prepare annealed barcode Bn

25 µL		2X annealing buffer
12.5 µL	[M] 100 micromolar (µM)	barcode Bn
12.5 µL	[M] 100 micromolar (µM)	ligation linker 2

Run the following program:

95 °C 00:05:00 , and cool to 12 °C , -0.1 °C/sec.

Note

Annealed barcodes can be stored at -20 °C

Prepare spatial barcode mix, for each channel

27 µL	10X T4 DNA ligase buffer
11 µL	T4 DNA ligase
5.4 µL	5% Triton X-100
11.58 µL	10X NEB buffer3.1
175 µL	Nuclease free water

Prepare spatial barcode mix, for each channel:

4 µL	ligation mix
1 µL	annealed barcode An (such as A1)

50 Apply the microfluidic chip A to the tissue slide to cover the region of interest.

Note

Ensure the microfluidic chip is dust free by applying tape on the microfluid chip and tissue slide to gently remove dust.

51 Image full chip A on the tissue slide with no clamp using Keyence microscopy with an 10× objective lens.

52 Load the slide into a clamp. Secure the clamp onto the slide, and tighten the screws.

53 Load the spatial barcode mix A to the inlets, and attach a vacuum gasket over the outlets.

54 Flow the chip until the solution has reached the outlet wells for all channels.

55 Remove the vacuum gasket and incubate the tissue slide at 37 °C for 00:30:00 . 30m

56 Suck the solution out of the inlet and outlet.

57 Dip the slide in RNase free water and air dry the slide.

In-tissue ligation with barcode B 10m

58 Follow the steps from 50 to step 57, and only need to replace the barcode A with barcode B..

59 Image the tissue slide using Keyence microscopy with an 10× objective lens.

Lysis

60 Prepare the reverse crosslinking buffer (for one tissue slide)

5 µL	1M Tris-HCl,pH 8.0
10 µL	10mM EDTA,pH 8.0
10 µL	10% SDS
4 µL	5M NaCl
2 µL	20mg/ml proteinase K
69 µL	Nuclese-free water

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

62 Add 100 µL reverse crosslinking buffer and cover the hole with Parafilm or tapes.

63 Incubate the tissue slide at 60 °C for 02:00:00 . 2h

64 Collect the lysate into a fressh PCR tube and Incubate the tissue slide at 60 °C for Overnight .

Pause point Store lysate at −80 °C.

65 Purify the lysate with Zymo DNA Clean and concentrator kit in a 5:1 ratio (sample : buffer).

66 Elute the purified DNA with 100 µL RNase free water.

gDNA and cDNA separation 10m

67 Prepare buffer:

2x Wash Buffer (2xW&B)

reagents	Final concentration	volume	volume	volume
1M Tris-HCl, pH8.0	10mM	2.5ul	5ul	10ul
5M NaCl	2M	100ul	200ul	400ul
0.5M EDTA, pH8.0	1mM	0.5ul	1ul	2ul
Nuclear-free water	2x588ul	147ul	294ml	588ul
Total		0.25ml	0.5ml	1ml
SUPERase IN inhibitor 20U/ul	0.375U/ul	6.25ul	12.5ul	
		1x reaction	2x reactions	5x reactions

1x Binding Wash buffer-Tween (1x TBW)

reagents	Final concentration	volume	volume	volume
1M Tris-HCl, pH8.0	5mM	5ul	25ul	50ul
5M NaCl	1M	200ul	1ml	2ml
0.5M EDTA,pH8.0	0.5mM	1ul	5ul	10ul
10% Tween 20	0.05%	5ul	25ul	50ul
Nuclear-free water		789ul	3.945ml	7.89ml
Total		1ml	5ml	10ml
SUPERase IN inhibitor 20U/ul	0.0275U/ul	1.38ul	6.875ul	

1x Tris-HCl Buffer (1xTris-HCl Buffer)

reagents	Final concentration	volume	volume	volume
1M Tris-HCl, pH8.0	10mM	5ul	10ul	20ul
10% Tween 20	0.1%	5ul	10ul	20ul
Nuclear-free water		490ul	980ul	2x980ul
Total		500ul	1ml	2ml
SUPERase IN inhibitor 20U/ul	0.05U/ul	1.25ul	2.5ul	5ul

68 Wash 40 µL Dynabeads™ MyOne™ Streptavidin C1 beads with 800 µL 1XTBW buffer.

69 Repeate step 68 for 2 times.

70 Resuspend the beads with 100 µL 2xBW buffer and the purified DNA from step 66.

71 Agitate the DNA-beads at Room temperature for 01:00:00 . 1h

72 Use a magnetic rack to separate beads (containing cDNA/ADT) and supernatant (containing gDNA).

RNA/ADT library construction

73 Add 400 µL of 1X TBW buffer to the seperated beads and resuspend.

74 Separate the beads from the supernatant on the magnetic rack for 1 min and remove the supernatant.

75 Repeate step 73-74.

76 Add 400 µL of 1XTris-HCl buffer to the seperated beads and resuspend.

77 Agitate beads for 00:05:00 at Room temperature . 5m

78 Separate the beads from the supernatant on the magnetic rack for 1 min and remove the supernatant.

79 Add 400 µL of RNase free water to the seperated beads.

80 Prepare TSO solution

22 µL	10mM dNTPs
44 µL	5X Maxima RT buffer
44 µL	20% Ficoll PM-400 solution
5.5 µL	[M] 100 micromolar (µM) TSO primer
11 µL	Maxima H Minus Reverse Transcriptase
5.5 µL	RNase Inhibitor
88 µL	RNase free water

81 Separate the beads from the supernatant on the magnetic rack for 1 min and remove the supernatant.

82 Add 200 µL TSO solution and gentlely shake the beads at Room temperature for 00:30:00 30m

83 Gentlely shake the beads at 42 °C for 01:30:00 1h 30m

84 Separate the beads from the supernatant on the magnetic rack for 1 min and remove the supernatant.

85 Add 400 µL of 1XTris-HCl buffer to the seperated beads and resuspend.

86 Agitate beads for 00:05:00 at Room temperature . 5m

87 Separate the beads from the supernatant on the magnetic rack for 1 min and remove the supernatant.

88 Add 400 µL of RNase free water to the seperated beads.

89 Prepare the PCR mixture

110 µL		2X KAPA HIFI HotStart Master Mix
8.8 µL	[M] 10 micromolar (µM)	Primer 1
8.8 µL	[M] 10 micromolar (µM)	Primer 2
1 µL	[M] 10 micromolar (µM)	Primer 3
91.4 µL		Nuclease Free water

90 Add 200 µL PCR mix to the separated beads. Splite the beads into 4 PCR tubes.

91 PCR thermocyling was performed as following:

Cycling conditions	
95°C	3 min
Then 5 cycles of:	
98°C	20 sec
65°C	45 sec
72°C	3 min
Hold at 4 °C	

92 Separate the beads from the supernatant on the magnetic rack for 1 min and transfer the supernant to a fresh tube (1.5 ml).

93 Add 10 µL 20X EvaGreen and splite into 5 qPCR tubes (46 µL for each tube).

94 Run with a qPCR machine with the following thermocycling conditions:

Cycling conditions	
95°C	3 min
Then 20 cycles of:	
98°C	20 sec
65°C	20 sec
72°C	3 min
72°C	5 min
Hold at 4 °C	

Once the qPCR signal began to plateau, reactions are stopped.

95 PCR reactions are purified using a 0.6x ratio of SPRI beads according to the manufacturer's protocol, and the cDNA is eluted with 20 µL Nuclease free water.

Note

Keep the supernatant for ADT library construction.

96 Confirm the quality and quantity of the cDNA library with the agilent tapestation.

Note

The sample can be stored at -20 °C

97 RNA library construction

Nextera XT DNA Library Preparation Kit is used for final RNA library construction.

98 **ADT library construction**

During SPRI cleanup (Step 95), the supernatant is saved and the short DNA derived from antibody oligos is purified with 2.0× SPRI beads:

- Transfer the supernatant to a new tube and add 1.4X SPRIselect reagent to obtain a final SPRI volume of 2X SPRI.
- purify the SPRI beads according to the manufacturer's protocol, and the beads are eluted with 50 µL Nuclease free water.
- Second time purification with 2.0X SPRI beads and he beads are eluted with 30 µL Nuclease free water.

ADT library amplification:
Prepare 100ul PCR reaction with purified ADT:

- 30 µL purified ADT fraction
- 50 µL 2x KAPA Hifi PCR Master Mix.
- 1.5 µL [M] 10 micromolar (µM) Nextera DNA Index.
- 1.5 µL [M] 10 micromolar (µM) P5 oligo.
- 17 µL Nuclease free water.

PCR program:
95°C 3 min
95°C 20 sec |
60°C 30 sec | ~ 6-10 cycles
72°C 20 sec |
72°C 5 min

PCR reactions are purified using a 1.6x ratio of SPRI beads according to the manufacturer's protocol, and the cDNA is eluted with 20 µL Nuclease free water.

Confirm the quality and quantity of the cDNA library with the agilent tapestation.

The sample can be stored at -20 °C

ATAC/nano-CUT&Tag library construction

99 During Step 72, the supernatant is saved and the gDNA is purified with Zymo DNA Clean and concentrator kit in a 5:1 ratio (sample : buffer).

The column is eluted with 45 µL Nuclease free water.

ATAC/nano-CUT&Tag **library amplification:**
Prepare 100ul PCR reaction with purified ADT:

- 45 µL purified ATAC/nano-CUT&Tag fraction
- 50 µL 2X NEB Master Mix.
- 2.5 µL [M] 10 micromolar (µM) Nextera DNA Index.
- 2.5 µL [M] 10 micromolar (µM) P5 oligo.

PCR program:
58˚C 5 min
72˚C 5 min
98˚C 30 sec
98˚C 10 sec |
60˚C 30 sec | ~ 13 cycles
72˚C 5 min

PCR reactions are purified using a 1.2x ratio of SPRI beads according to the manufacturer's protocol, and the final gDNA library is eluted with 20 µL Nuclease free water.

Confirm the quality and quantity of the library with the agilent tapestation.

The sample can be stored at -20 °C