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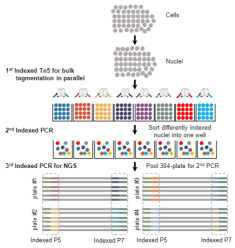
IT-scATAC-seq



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We use this protocol and it's working in many settings.

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The distribution of this protocol is solely intended for non-commercial academic research purposes, and a patent (PCT/CN2024/106967) associated with it has been submitted.

Abstract

Single-cell ATAC-seq (scATAC-seq) allows for detailed mapping of chromatin accessibility but often faces challenges related to throughput, cost, and equipment demands. In this study, we introduce indexed Tn5 tagmentation-based scATAC-seq (IT-scATAC-seq), a semi-automated, economical, and scalable method that utilizes indexed Tn5 transposomes along with a three-round barcoding strategy. This workflow can prepare libraries for up to 10,000 cells in just one day, lowers the cost per cell to around $0.01, and preserves strong data resolution.

Image Attribution

Microsoft PPT and image J.

Guidelines

Semi-automated IT-scATAC-seq profiles cell-specific chromatin accessibility in differentiation and peripheral blood populations

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Materials

1) E. coli C3013 cells (NEB, C2527I)
2) Chitin resin (NEB, S6651S)
3) Digitonin (Sigma-Aldrich, D141-500MG)
4) AMPure XP (Agencourt, A63880)
5) Proteinase K (NEB, P8111S)
6) High-Fidelity 2X PCR Master Mix (NEB, M0494L)
7) Exo I (NEB, M0293S)
8) Echo Qualified 384-Well Polypropylene Microplate 2.0 (LabCyte, PP-0200)
9) Hard-Shell 384-well PCR plate (Bio-Rad, HSR4805)
10) 0.2 ml PCR tubes
11) 1.5 ml Eppendorf tubes
12) Eppendorf ThermoMixer
13) 384-well PCR thermal cycler
14) Magnetic rack (Thermofisher)
15) 1 mL pipette (Eppendorf)
16) 100 µL pipette (Eppendorf)
17) 20 µL pipette (Eppendorf)
18) 2.5 µL pipette (Eppendorf)
19) 15 mL tube

Buffer preparation

1 In-house prepared Tn5: 30μM

ATAC-Resuspension buffer (RSB): Mix 500μL 1 M Tris-HCl (pH 8.0), 100μL 5 M NaCl, 150μL 1 M MgCl2, and 49.25 ml H2O for 50 mL omni-ATAC RSB.

ATAC-RSB-Hypotonic buffer: Mix 960 μL ATAC-RSB buffer with 10μL 10% Tween 20, 10μL 10% NP40 and 10μL 1% Digitonin and 10μL proteinase inhibitor.

ATAC-RSB wash buffer: Mix 990 μLATAC-RSB buffer with 10μL 10% Tween20.

Resuspension buffer: Mix 330μL PBS, 10μL 10% Tween-20, 10μL 1% Digitonin, 50μL 10% BSA with
600μL H2O.

5xTAPS-DMF buffer: Mix 20μL 1M TAPS-NaOH pH 8.2, 25μL 1M MgCl2, 500μL DMF and 455μL H2O
for 1 ml 5xTAPS-DMF buffer.

STOP buffer**:** 1xPBS buffer, 100mM EDTA, 0.5% BSA.

Lysis buffer: 0.2% SDS, 10mM Tris-HCl, 10mM NaCl. Mix 200μL 1 M Tris-HCl pH8.0, 40μL 5 M NaCl, 400μL 10% SDS and 19.4 ml ddH2O for 20 ml buffer, store at R.T. Freshly add 0.2 mg/ml Proteinase into lysis buffer and freeze the plate at-20^{o}C after Echo distribution.

Indexed Tn5 assmbly and quality control

2 Dissolve the indexed adapters and Tn5 reverse adapters with annealing buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 2mM EDTA) to make 200μM stock.

	Tn5-Rve	p-CTGTCTCTTATACACATCT
	Q501	GGCGGTAGGCGTGCTCCTAGCGCTAGATGTGTATAAGAGACAG
	Q502	GGCGGTAGGCGTGCTCTCGATATCAGATGTGTATAAGAGACAG
	Q503	GGCGGTAGGCGTGCTCCGTCTGCGAGATGTGTATAAG

		AGACAG
	Q504	GGCGGTAGGCGTGCTCTA CTCATAAGATGTGTATAAG AGACAG
	Q505	GGCGGTAGGCGTGCTCCG CTATGTAGATGTGTATAAGA GACAG
	Q506	GGCGGTAGGCGTGCTCTA TCGCACAGATGTGTATAAG AGACAG
	Q701	CCAACACCCGTGCGCTGG TGAATATAGATGTGTATAAG AGACAG
	Q702	CCAACACCCGTGCGCTGA CAGGCGCAGATGTGTATAA GAGACAG
	Q703	CCAACACCCGTGCGCTGC ATAGAGTAGATGTGTATAAG AGACAG
	Q704	CCAACACCCGTGCGCTGT GCGAGACAGATGTGTATAA GAGACAG

Table 1. Sequences of Tn5 assembly associated adapters.

3 Prepare 15µLof individual Q5XX and Q7XX adapters with equal Tn5-reverse adapter in 200µL PCR tube and anneal in a thermocycler as follows: 98 °C for 10 min, and slowly cool down to 23 °C with −0.1 °C/s. Mix the annealed adapter with 100µL 30µM Tn5 and 70µL coupling buffer (100mM HEPES-NaOH, 500mM NaCl, 50%
v/v Glycerol, 0.5mM EDTA, 2mM DTT), and incubate in thermomixer at 25°C, 1000 rpm for one hour. The indexed Tn5 transposome was prepared by mixing 20µL of the paired two Tn5-adapters with 80µL coupling buffer and the resulting Tn5 transposome complex were 5µM.

	IT-ATAC-Index#1	Q501& Q701	IT-ATAC-Index#13	Q504& Q701

	IT-ATAC-Index#2	Q501& Q702	IT-ATAC-Index#14	Q504& Q702
	IT-ATAC-Index#3	Q501& Q703	IT-ATAC-Index#15	Q504& Q703
	IT-ATAC-Index#4	Q501& Q704	IT-ATAC-Index#16	Q504& Q704
	IT-ATAC-Index#5	Q502& Q701	IT-ATAC-Index#17	Q505& Q701
	IT-ATAC-Index#6	Q502& Q702	IT-ATAC-Index#18	Q505& Q702
	IT-ATAC-Index#7	Q502& Q703	IT-ATAC-Index#19	Q505& Q703
	IT-ATAC-Index#8	Q502& Q704	IT-ATAC-Index#20	Q505& Q704
	IT-ATAC-Index#9	Q503& Q701	IT-ATAC-Index#21	Q506& Q701
	IT-ATAC-Index#10	Q503& Q702	IT-ATAC-Index#22	Q506& Q702
	IT-ATAC-Index#11	Q503& Q703	IT-ATAC-Index#23	Q506& Q703
	IT-ATAC-Index#12	Q503& Q704	IT-ATAC-Index#24	Q506& Q704

Table 2. Index number of Tn5 for IT-scATAC-seq.

4 Prepare 1 µL 300 ng/µL genomic DNA, 4 µL 5xTAPS-DMF buffer (50 mM TAPS-NaOH pH 8.2, 25 mM MgCl2, 50% DMF), 13 µL H2O, 2 µL assembled Tn5. Incubate at 55 °C for 10 min, then add 2 µL 10X STOP buffer (2% SDS, 40 mM EDTA) and quench at 37^{o}C 15min to dissociate Tn5 from DNA. Add 5 µL 6x Loading dye and run 1.5% DNA gel. The majority of tagmentated DNA sizes were less than 1,000bp, indicating the assembled transposases are qualified for downstream experiments.

Step-by-step protocol.

5 **Collect cells**. For culture cells: Harvest the cells with digestion enzymes and wash with 1x PBS buffer in 1.5mL Low bond tube; for cryopreserved cells, thaw the cells quickly at 37 °C water batch and transfer to pre-warmed medium and centrifuge 1000g 5min, check the cell viability (>95%) and density, and wash with 1x PBS buffer in 1.5mL Low bond tube; for tissue dissected cells, 0.1-0.2% formaldehyde crosslinking at room-temperature for 10min is recommended.

6 **Nuclei isolation by OmniATAC buffer**. Discard the PBS and resuspend the cells (250,000 for 5 indexed transposition reactions) with 100µL ATAC-RSB-Hypotonic buffer, lyse on ice for 5min, add 1mL ATAC-RSB wash buffer and up and down 5 times before spinning down by 500g for 10min.

7 **Indexed Tn5 tagmentation**. Discard the buffer avoiding disrupt the nuclei, and resuspend with 100 µL 0.33X PBS containing 0.1% Tween 20 and 0.01% Digitonin. Aliquot 20 µL resuspended nuclei (50,000 nuclei) to five Low bond tubes, followed by adding 20µL 5×Tagmentation buffer and 5µL indexed Tn5. Record the used indexed Tn5 number, and gently pipetting up and down 10 times and perform the tagmentation assay on a thermomixer at 37 °C 850 rpm for 30min. Add 500 µL STOP buffer to stop the reaction for 5min on ice.

8 **Sorting plate preparation.** Distribute 350nL lysis buffer to each well using liquid handling system Echo 550. Spin down the lysis buffer 3,000g for 2min. The sorting plates can be prepared just before usage or frozen at -20°C for ready use.

9 **Single nuclei sorting**. Add 6µL 100 µg/µL DAPI to stain the tagmentated nuclei and transfer the nuclei to flow tubes for sorting. A non-stained nuclei can be prepared in parallel for DAPI negative calibration. Different index-tagmentated nuclei are sorted into the same well of 384-plate.
NOTE: After sorting, the plates can be frozen at -20°C until lysis.

10 **Nuclei lysis and 1st barcoded PCR.** Spin down the sorted nuclei to lysis buffer and incubate at 37°C for 15min. Add 100 nL 10% Triton X-100 to quench the 0.2% SDS in lysis buffer. Add 25nL indexed H5XX and 25nL indexed H7XX, as well as 500nL NEB

High-Fidelity 2X PCR Master Mix, to each well and spin down. The first PCR is carried out with 72°C 5min, 98 °C 30s; 12 cycles of 98 °C 20 s, 63°C 30s,72 °C 1min; 72 °C 5min, 4 °C hold.

	H501	ACTCTTTCCCTACACGACG CTCTTCCGATCTTAGATCG CGGCGGTAGGCGTGCTC
	H502	ACTCTTTCCCTACACGACG CTCTTCCGATCTCTCTCTA TGGCGGTAGGCGTGCTC
	H503	ACTCTTTCCCTACACGACG CTCTTCCGATCTTATCCTC TGGCGGTAGGCGTGCTC
	H504	ACTCTTTCCCTACACGACG CTCTTCCGATCTAGAGTAG AGGCGGTAGGCGTGCTC
	H505	ACTCTTTCCCTACACGACG CTCTTCCGATCTGTAAGGA GGGCGGTAGGCGTGCTC
	H506	ACTCTTTCCCTACACGACG CTCTTCCGATCTACTGCAT AGGCGGTAGGCGTGCTC
	H507	ACTCTTTCCCTACACGACG CTCTTCCGATCTAAGGAGT AGGCGGTAGGCGTGCTC
	H508	ACTCTTTCCCTACACGACG CTCTTCCGATCTCTAAGCC TGGCGGTAGGCGTGCTC
	H509	ACTCTTTCCCTACACGACG CTCTTCCGATCTCGTCTAA TGGCGGTAGGCGTGCTC
	H510	ACTCTTTCCCTACACGACG CTCTTCCGATCTTCTCTCC GGGCGGTAGGCGTGCTC
	H511	ACTCTTTCCCTACACGACG CTCTTCCGATCTTCGACTA GGGCGGTAGGCGTGCTC
	H512	ACTCTTTCCCTACACGACG CTCTTCCGATCTTTCTAGC TGGCGGTAGGCGTGCTC
	H513	ACTCTTTCCCTACACGACG CTCTTCCGATCTCCTAGAG TGGCGGTAGGCGTGCTC

	H514	ACTCTTTCCCTACACGACG CTCTTCCGATCTGCGTAAG AGGCGGTAGGCGTGCTC
	H515	ACTCTTTCCCTACACGACG CTCTTCCGATCTCTATTAA GGGCGGTAGGCGTGCTC
	H516	ACTCTTTCCCTACACGACG CTCTTCCGATCTAAGGCTA TGGCGGTAGGCGTGCTC
	H701	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCGCCT TACCAACACCCGTGCGCT G
	H702	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTCTAGTA CGCCAACACCCGTGCGCT G
	H703	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTTTCTGC CTCCAACACCCGTGCGCT G
	H704	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTGCTCAG GACCAACACCCGTGCGCT G
	H705	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTAGGAGT CCCCAACACCCGTGCGCT G
	H706	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTCATGCC TACCAACACCCGTGCGCT G
	H707	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTGTAGAG AGCCAACACCCGTGCGCT G
	H708	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTCCTCTC TGCCAACACCCGTGCGCT G
	H709	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTAGCGTA GCCCAACACCCGTGCGCT G
	H710	GACTGGAGTTCAGACGTGT GCTCTTCCGATCTCAGCCT

		CGCCAACACCCGTGCGCTG
	H711	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTCTTCCAACACCCGTGCGCTG
	H712	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTCTACCCAACACCCGTGCGCTG
	H713	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATGAGCCCAACACCCGTGCGCTG
	H714	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGAGATCCAACACCCGTGCGCTG
	H715	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGCGAGTCCAACACCCGTGCGCTG
	H716	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTAGCTCCCCAACACCCGTGCGCTG
	H717	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACTACGCCCAACACCCGTGCGCTG
	H718	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGCTCCGCCAACACCCGTGCGCTG
	H719	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCGTACCAACACCCGTGCGCTG
	H720	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCGCATCCAACACCCGTGCGCTG
	H721	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCGCTACCAACACCCGTGCGCTG
	H722	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCTCA

		GTCCAACACCCGTGCGCTG
	H723	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTTAGGCCAACACCCGTGCGCTG
	H724	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGATCGCCAACACCCGTGCGCTG

Table 3. The first round of PCR primers.

11 **PCR purification and adapter removal.** Added 6μL PB buffer (Qiagen) to each well using 24-well multichannel pipette, pooled the PCR product by inverting the plate and centrifuge to a
container, followed by purification using MinElute column (Qiagen) through pumping and finally elute with 50μLnuclease-free H2O. The undesired fragments, primers and adapters were removed by Exo I digestion (NEB), 1.0x AMPure XP beads purification and eluted with 25μL nuclease-free H2O.

12 **Truseq adapter addition by 2nd PCR.** Prepare 23μL eluted 1st PCR product, 2μL 10μM Truseq T5XX/T7XX and 25μL NEB High-Fidelity 2X PCR Master Mix in 200μLPCR tube. The 2nd PCR is carried out following 98 °C 30s; 2-3 cycles of 98 °C 20s, 63°C 30s, 72 °C 1min; 72 °C 5min, 4 °C hold.

	T501	AATGATACGGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCCTACACGACGC
	T502	AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCCCTACACGACGC
	T503	AATGATACGGCGACCACCGAGATCTACACCCTATCCTACACTCTTTCCCTACACGACGC
	T504	AATGATACGGCGACCACCGAGATCTACACGGCTCTGAACACTCTTTCCCTACACGACGC
	T505	AATGATACGGCGACCACCGAGATCTACACAGGCGAAG

		ACACTCTTTCCCTACACGACGC
	T506	AATGATACGGCGACCACCGAGATCTACACTAATCTTAACACTCTTTCCCTACACGACGC
	T507	AATGATACGGCGACCACCGAGATCTACACCAGGACGTACACTCTTTCCCTACACGACGC
	T508	AATGATACGGCGACCACCGAGATCTACACGTACTGACACACTCTTTCCCTACACGACGC
	T701	CAAGCAGAAGACGGCATACGAGATCGAGTAATGTGACTGGAGTTCAGACGTGT
	T702	CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTGGAGTTCAGACGTGT
	T703	CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTGGAGTTCAGACGTGT
	T704	CAAGCAGAAGACGGCATACGAGATGGAATCTCGTGACTGGAGTTCAGACGTGT
	T705	CAAGCAGAAGACGGCATACGAGATTTCTGAATGTGACTGGAGTTCAGACGTGT
	T706	CAAGCAGAAGACGGCATACGAGATACGAATTCGTGACTGGAGTTCAGACGTGT
	T707	CAAGCAGAAGACGGCATACGAGATAGCTTCAGGTGACTGGAGTTCAGACGTGT
	T708	CAAGCAGAAGACGGCATACGAGATGCGCATTAGTGACTGGAGTTCAGACGTGT
	T709	CAAGCAGAAGACGGCATACGAGATCATAGCCGGTGACTGGAGTTCAGACGTGT
	T710	CAAGCAGAAGACGGCATACGAGATTTCGCGGAGTGACTGGAGTTCAGACGTGT
	T711	CAAGCAGAAGACGGCATACGAGATGCGCGAGAGTGA

		CTGGAGTTCAGACGTGT
	T712	CAAGCAGAAGACGGCATA CGAGATCTATCGCTGTGAC TGGAGTTCAGACGTGT

Table 4. Sequences of TrueSeq primers.

13 **Library purification and NGS**. Purify the library via AMPure XP beads double selection (0.50x/0.35x)
to remove primer dimers and large size fragments, and elute with 20-40μL nuclease-free H2O. The concentration is quantified with Qubit 4.0 and we usually get 5-50 ng/μL (cell-context
dependent) of the final library and sent for NGS.

Library quality control

14

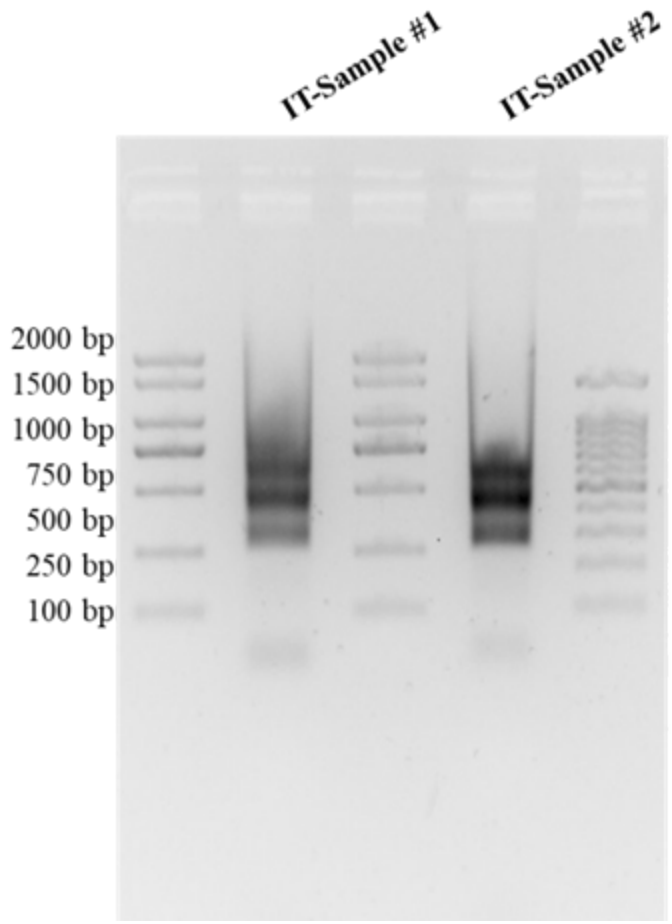

Figure 1.DNA electrophoresis of IT-scATAC-seq library.

15

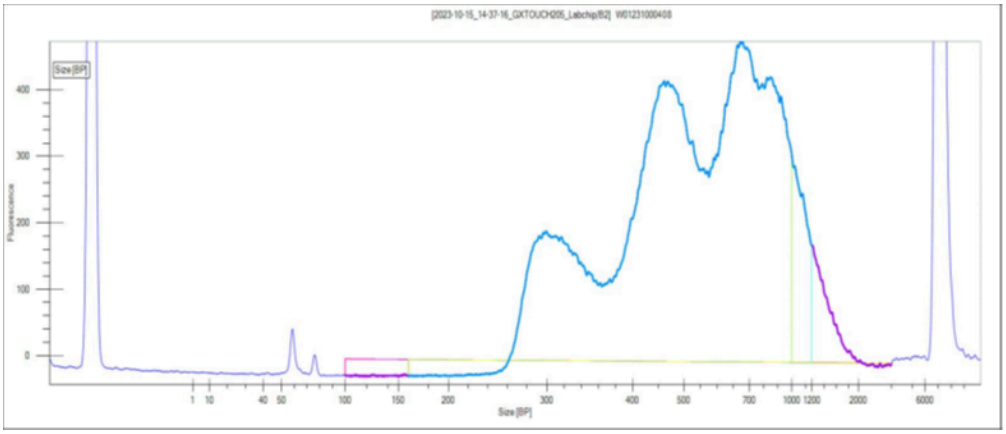

Figure 2.Representative of fragments distribution from a good IT-scATAC-seq library.

Protocol references

The Tn5 purification and assembly refers to Picelli, S. *et al.* Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome Res* **24**, 2033-40 (2014).

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