

Feb 25, 2019

In devel.

## scTHS-seq

Kun Zhang<sup>1</sup>, Brandon Sos<sup>1</sup>, Dinh Diep<sup>1</sup>, Thu E Duong<sup>1</sup>

<sup>1</sup>University of California, San Diego

dx.doi.org/10.17504/protocols.io.xf8fjrw



#### ABSTRACT

Single cell transposome hypersensitive site sequencing (scTHS-seq) combined transposome hypersensitive site sequencing (THS-seq) with combinatorial cellular indexing using customized barcoded transposomes. Compared to a similar method, ATAC-seq, THS-seq have improved coverage of distal enhancer sites which is due to the utilization of an engineered super-mutant Tn5 transposase as well as in vitro transcription to perform linear amplification from a single insertion site of the Tn5 transposase instead of two insertion sites required in ATAC-seq. scTHS-seq was used to generate single nucleus accessibility profile of 36,869 cells with an average of 10,168 unique reads per cell that are also associated with a predicted accessible region. The doublet rate of scTHS-seq is estimated to be between 20-25% which is comparable to similar combinatorial indexing methods.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://www.nature.com/articles/nbt.4038

PROTOCOL STATUS

### In development

We are still developing and optimizing this protocol

### MATERIALS

NAME ~		CATALOG #	<b>VENDOR</b> $\vee$
HiScribe T7 High Yield RNA Synthesis Kit - 50 rxns		E2040S	New England Biolabs
Taq 5X Master Mix - 500 rxns		M0285L	New England Biolabs
Pierce™ Dimethylformamide (DMF), Sequencing grade		20672	Thermo Fisher Scientific
2x Kapa HiFi Hotstart Readymix		KK2602	Kapa Biosystems
Tango Buffer		BY5	Thermo Fisher Scientific
Ultrapure SMART MMLV Reverse Transcriptase for RT-PCR		639524	Clontech
Advantage® UltraPure dNTP Combination Kit (100 mM each dNTP)		639125	Clontech
RNAse H		Y9220L	Enzymatics
KAPA Pure Beads (60 mL)		07983298001	Roche
twin.tec PCR Plate 96 LoBind semi-shirted clear 25 pcs.		30129504	Eppendorf
STEPS MATERIALS			
NAME ~	CATALOG #		<b>VENDOR</b> $\vee$
KAPA Pure Beads (60 mL)	07983298001		Roche
Taq 5X Master Mix - 500 rxns	M0285L		New England Biolabs
KAPA Pure Beads (60 mL)	07983298001		Roche
KAPA Pure Beads (60 mL)	07983298001		Roche

NAME ×	CATALOG # ~	VENDOR ~
KAPA Pure Beads (60 mL)	07983298001	Roche
KAPA Pure Beads (60 mL)	07983298001	Roche
KAPA Pure Beads (60 mL)	07983298001	Roche

MATERIALS TEXT

For purification of engineered super-mutant Tn5-059, see previous publications (<a href="https://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-0882-7">https://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-0882-7</a>) for details.

# Tagmentation Make 10 mL 75% glycerol with 0.3% tritonX-100 **■9.45** g Glycerol (100%) **300** μl TritonX-100, 10% ■2200 µl nuclease free water Make 3.045 mL transposons dilution buffer **2345** μl 75% glycerol/0.3% TritonX-100 **■700 μl Qiagen EB** Make 1000 uL transposase dilution buffer **□667 µl** 75% glycerol/0.3% TritonX-100 **■1** μl DTT, 1M **□**6.25 µl Tris-HCl, 4M, pH 7.5 ■325.75 µl nuclease free water Make 10 mL of 1.5X D6 buffer ■1500 µl Tango Buffer, 10X **■2400 µl DMF, 100%** ■6100 µl nuclease free water Make 10 mL of 1X lysis buffer 5 **25 µl** Tris-HCl, 4M, pH 7.5 ■20 µl Sodium chloride, 5M ⊒15 μl Magnesium chloride, 2M **100** μl IGEPAL-630, 10% **■2000 μl BSA, 10%**

**■1000 µl PBS, 10X** 

```
■6840 µl nuclease free water
      Finally, add 1 protease inhibitor tablet (EDTA-free).
      Make 10 mL of 2X FACS buffer
       ■1000 μl PBS, 10X
       ■2000 μl BSA, 10%
       ■80 μl EDTA, 0.5M
       ■6920 µl nuclease free water
      Generate 384 annealed transposons

□7 µl Indexed Tn5 top strand (100uM)

       □7 μl 5' phosphorylated mosaic end sequence bottom strand (100μM)
      Combine top and bottom strand oligos in a PCR tube. In a thermocycler, incubate for 2 minutes at 🐧 95 °C then ramp to 🐧 14 °C at
      a rate of § -0.1 °C per second.
 8
      Dilute annealed transposons

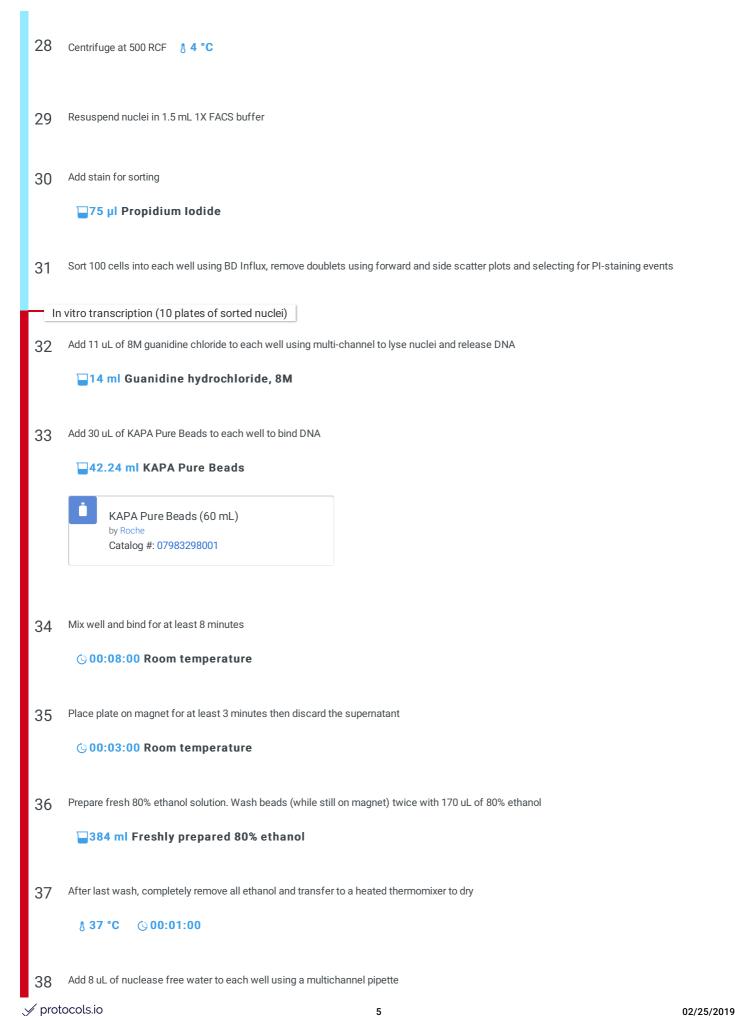
□1 µl Annealed transposons

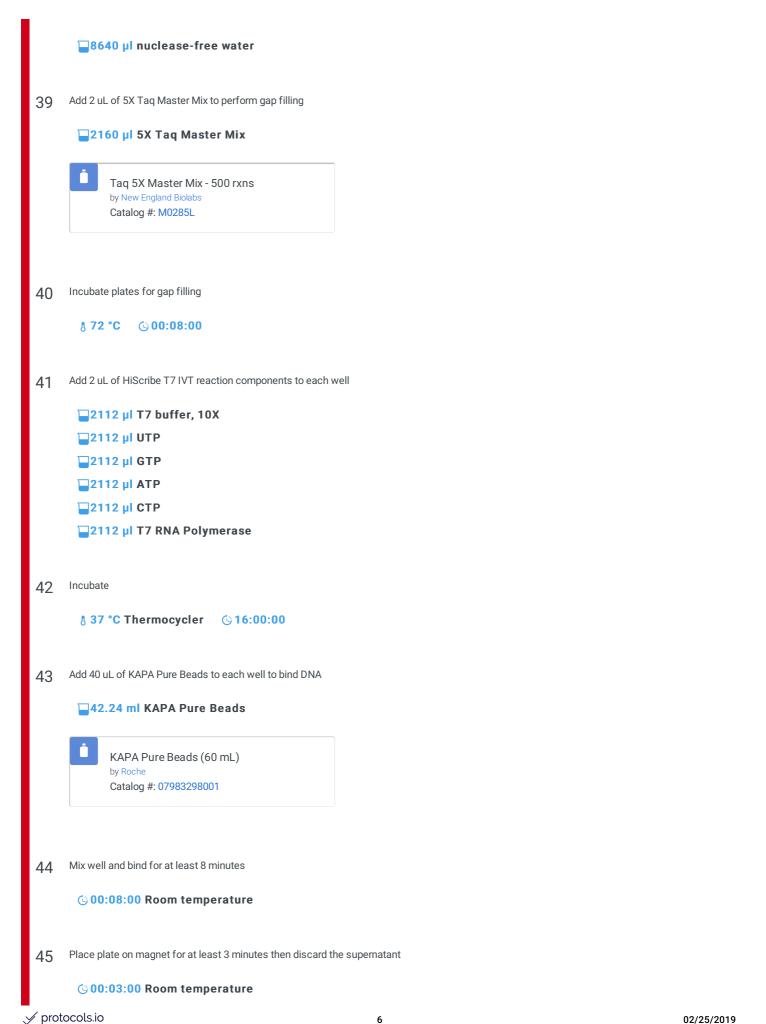
       ■6.7 µl transposon dilution buffer
      Make 420 uL of 4.8 uM transposase Tn5-059
       252 µl transposase, Tn5-059, 8 uM
       ■168 μl transposase dilution buffer
     Load Tn5-059
10
       ■1 µl 4.8 uM transposase Tn5-059
       1 μl diluted annealed transposons (6.5 μM)
11
     Incubate
        § 22 °C Thermocycler
                                 © 00:30:00
12
      Wash nuclei or cells suspension with ice-cold PBS
     Count cells
13
     Pellet 400,000 cells / nuclei by centrifuging at 330 RCF (cells) or 900 RCF (nuclei)
```

Resuspend 400,000 cells/nuclei in lysis buffer. Use 100 uL per 100,000 cells/nuclei 15 Incubate 16 § 4 °C On ice **(900:05:00** Pellet by centrifuging at 900 RCF 17 18 Resuspend nuclei in 100 uL 1.5X D6 buffer initially. Count and add more 1.5X D6 buffer to create ~250 nuclei/uL Aliquot 4.0 uL of cells in 1.5X D6 buffer into wells containing 2.0 uL of prepared transposomes 19 Incubation without shaking 20 § 37 °C Thermocycler **© 00:30:00** 21 Terminate the tagmentation reaction with EDTA **■**4 μl EDTA, 50 mM 22 Incubate § 37 °C Thermocycler **© 00:15:00** Store cells at -20C overnight or until ready to sort 23 FACS (BD Influx) Coat 12 x 200 uL, 1 x 15 mL conical using 1X FACS buffer 24 () 01:00:00 Let tubes sit in FACS buffer 25 Add 10 uL PBS to each well of 10 to 15 x 96-wells plate for sorting B Cover and store all plates on ice. 26 Add 1 volume (10 uL) ice cold 2X FACS buffer to each sample well with cells in tagmentation mix When 15 mL conical have been coated for 1 hr, pool all samples together using multichannel pipette and adding to 12 x 200 uL tubes

02/25/2019

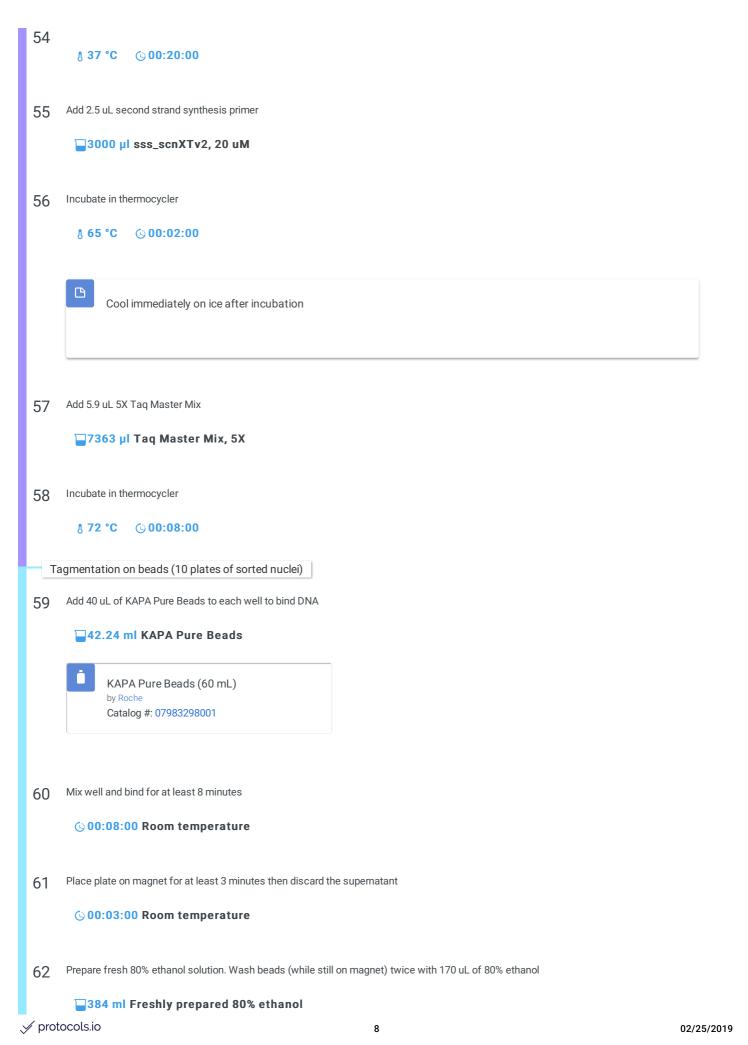
✓ protocols.io





```
Prepare fresh 80% ethanol solution. Wash beads (while still on magnet) twice with 170 uL of 80% ethanol
46
       384 ml Freshly prepared 80% ethanol
      After last wash, completely remove all ethanol and transfer to a heated thermomixer to dry
47
       8 37 °C © 00:01:00
     Add 9 uL of nuclease free water to each well using a multichannel pipette
48
       ■9720 µl nuclease-free water
 cDNA synthesis (10 plates of sorted nuclei)
     Add 2.5 uL of random hexamers
49
       ■3000 μl random hexamers, 20 uM
     Incubate
50
        § 70 °C Thermocycler
                               © 00:03:00
             Cool immediately on ice after.
     Add 9.5 uL of MMLV RT mixture
51
       ■4224 µl First strand buffer, 5X
       ■2112 μl Advantage UltraPure dNTPs, 100 uM each
       ■2112 μl DTT, 100mM
       ■528 µl MMLV RT
     Incubate in a thermocycler
52
        8 22 °C © 00:10:00
        A 42 °C
                 © 01:00:00
                  © 00:10:00
        § 70 °C
     Add 1 uL RNase H
53
       ■1127.52 µl nuclease-free water
       ■125.28 μl RNase H, 5 Units/uL
```

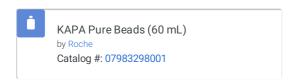
Incubate in thermocycler



After last wash, completely remove all ethanol and transfer to a heated thermomixer to dry 63 § 37 °C © 00:01:00 Add 4 uL of 1.5X tagmentation buffer to each well using a multichannel pipette 64 ■3172 µl nuclease-free water ■780 μl Tango Buffer, 10X **■1248** μl DMF, 100% 65 Generate annealed transposons **■40** µl i7 top strand (100uM) □40 μl 5' phosphorylated mosaic end sequence bottom strand (100uM) Combine top and bottom strand oligos in a PCR tube. In a thermocycler, incubate for 2 minutes at 🐧 95 °C then ramp to 🐧 14 °C at a rate of § -0.1 °C per second. 66 Dilute annealed transposons ■74 μl Annealed transposons ■1026 µl transposon dilution buffer Make 420 uL of 2.4 uM transposase Tn5-059 67 ■330 µl transposase, Tn5-059, 8 uM ☐770 µl transposase dilution buffer 68 Load Tn5-059 **□1100 μl 2.4 uM transposase Tn5-059** ■1100 µl diluted annealed transposons (6.5 uM) 69 Incubate § 22 °C Thermocycler **© 00:30:00** 70 Add 2 uL of loaded transposomes to each well



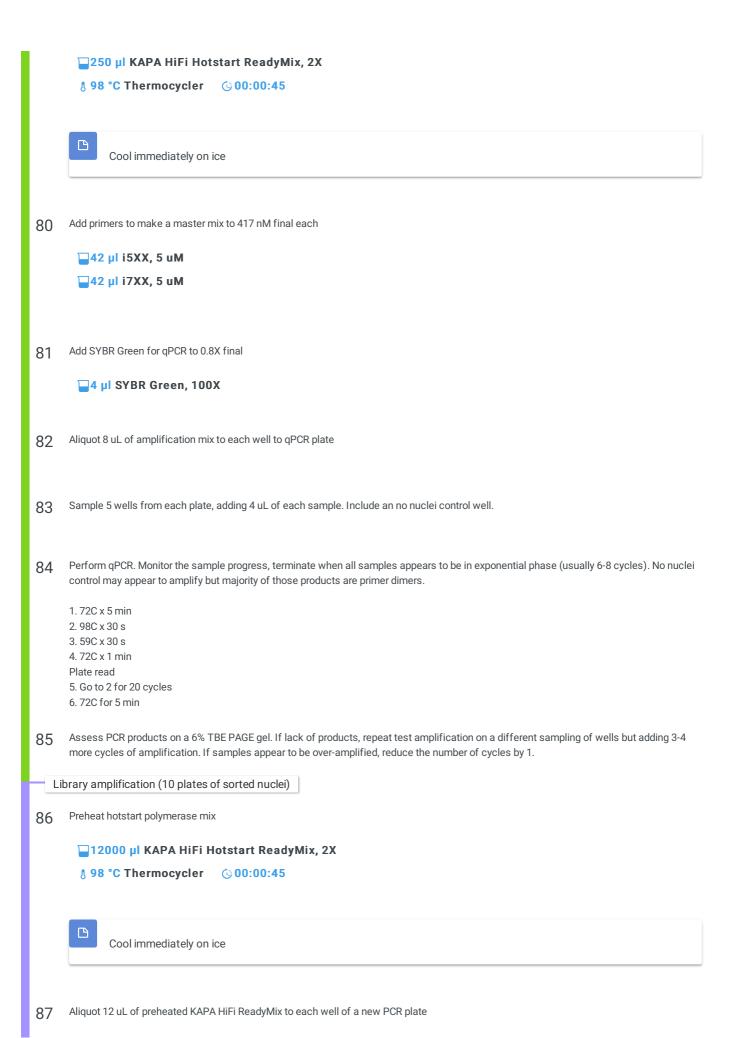
- 72 Add 6 uL guanidine hydrochloride
  - **■7488** μl Guanidine hydrochloride, 8M
- 73 Add 30 uL of KAPA Pure Beads to each well to bind DNA
  - **31.68 ml** KAPA Pure Beads



- 74 Mix well and bind for at least 8 minutes
  - **७** 00:08:00 Room temperature
- 75 Place plate on magnet for at least 3 minutes then discard the supernatant
  - **७** 00:03:00 Room temperature
- 76 Prepare fresh 80% ethanol solution. Wash beads (while still on magnet) twice with 170 uL of 80% ethanol
  - **384** ml Freshly prepared 80% ethanol
- 77 After last wash, completely remove all ethanol and transfer to a heated thermomixer to dry
  - § 37 °C © 00:01:00
- 78 Add 16 uL nuclease free water
  - 21.6 ml nuclease-free water

Test amplification (10 plates of sorted nuclei)

79 Preheat hotstart polymerase mix



√ protocols.io 11 02/25/2019

Aliquot 2 uL of 16 different s5XX primers to each set of 2 plates (one per row) 88 150 µl each s5XX primer, 5 uM 89 Aliquot 2 uL of 12 different s7XX primers to each column of every plate (one per column) ■100 µl s7XX primer, 5 uM Transfer 8 uL of sample to each well of PCR plate 90 Perform PCR using the number of cycles determined previously with test PCR 91 1. 72C x 5 min  $2.98C \times 30 s$  $3.59C \times 30 s$ 4. 72C x 1 min 5. Go to 2 for XX cycles 6. 72C for 5 min Pool 4 uL of each well from 2 plates with non-overlapping s5XX primers. Final volume is ~800 uL per pool. 5 pools total 92 Add 800 uL of KAPA Pure Beads to each pool to bind DNA 93 ■3 ml KAPA Pure Beads KAPA Pure Beads (60 mL) by Roche Catalog #: 07983298001 Mix well and bind for at least 8 minutes 94 **© 00:08:00** Room temperature 95 Place plate on magnet for at least 3 minutes then discard the supernatant **© 00:03:00** Room temperature Prepare fresh 80% ethanol solution. Wash beads (while still on magnet) twice with 1000 uL of 80% ethanol 96 12 ml Freshly prepared 80% ethanol

- 97 After last wash, completely remove all ethanol and transfer to a heated thermomixer to dry

  8 37 °C © 00:01:00

  98 Add 20 uL of nuclease free water to each pool

  100 μl nuclease-free water

  99 Quantify each pool using dsDNA HS Qubit
- Perform gel size selection for 220-1000 bp using 6% TBE PAGE. Use 5-wells gel, and loading  $\sim$ 1 ug of library per well.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited