**Tagmentation DNA with In-house Tn5**

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Note: 1) This protocol can be used for less 1ng input DNA and single cell DNA.

2) Up to 4 barcodes can be added into the library.

**1, Oligo preparation \*\*\*\*\*Note all of these oligos need to be HPLC purified\*\*\*\*\***

**For 4 barcodes library:**

MEs barcodes:

MEs-i5: 5’-TCG TCG GCA GCG TCT CCA CGC NNN NNN NNG CGA TCG AGG ACG GCA GAT GTG TAT AAG AGA CAG-3’

MEs-i7: 5’-GTC TCG TGG GCT CGG CTG TCC CTG TCC NNN NNN NNC ACC GTC TCC GCC TCA GAT GTG TAT AAG AGA CAG-3’

MEs-rev: 5’-/Phos/CTG TCT CTT ATA CAC ATC T-3’

PCR barcodes:

PCR-i5: 5’-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACN NNN NNN NNN TCG TCG GCA GCG TC-3’

PCR-i7: 5’-CAA GCA GAA GAC GGC ATA CGA GAT NNN NNN NNN NGT CTC GTG GGC TCG G-3’

**For 2 barcodes library (same as Nextera Kit): \*\*\*\*\*Note all of these oligos need to be HPLC purified\*\*\*\*\***

MEs Barcodes:

MEs-A: 5’- TCG TCG GCA GCG TC**A GAT GTG TAT AAG AGA CAG**-3’

MEs-B: 5’- GTC TCG TGG GCT CGG **AGA TGT GTA TAA GAG ACA G-**3’

MEs-rev: 5’-/Phos/**CTG TCT CTT ATA CAC ATC T**-3’

PCR barcodes:

PCR-i5: 5’-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACN NNN NNN NTC GTC GGC AGC GTC-3’

PCR-i7: 5’-CAA GCA GAA GAC GGC ATA CGA GAT NNN NNN NNG TCT CGT GGG CTC GG-3’

NNN: barcodes

AGAT…: Mosaic End

**Oligo Resuspension solution**

Resuspend Oligos in 1X Anneal Buffer

Make 10X Anneal buffer: 100 mM Tris-HCl, 10 mM EDTA, 250 mM NaCl, PH 8.0 Store @4°C

Dissolve MEs oligos into 100 μM with 1x Anneal buffer.

Dissolve PCR oligos into 100 μM with H2O for stock, dilute to 10 μM for use.

**Anneal adaptor: STOCK Working Solution**

MEs-i5 (100 μM) + MEs-rev (100 μM) 🡺 MEs-i5A (50 μM) 🡪 dilute to 1.25 μM or 2.5 μM with 1X Anneal buffer

MEs-i7 (100 μM) + MEs-rev (100 μM) 🡺 MEs-i7A (50 μM) 🡪 dilute to 1.25 μM or 2.5 μM with 1X Anneal buffer

Or

**STOCK Working Solution**

MEs-A (100 μM) + MEs-rev (100 μM) 🡺 MEs-AA (50 μM) 🡪 dilute to 1.25 μM with 1X Anneal buffer

MEs-B (100 μM) + MEs-rev (100 μM) 🡺 MEs-BA (50 μM) 🡪 dilute to 1.25 μM with 1X Anneal buffer

Anneal 50uM stocks and store leftovers at -20°C

Program: 95 °C for 3 min, then cooling to 20 °C with 0.1°C/s. Lid 105°C

**2, In-house Tn5 assembly.**

MEs-i5A (1.25 μM): 1 μl

MEs-i5B (1.25 μM): 1 μl

100% Glycerol: 1.3 μl

Tn5 (Lucigen): 1 μl

Total: 4 μl Incubate: 37 °C for 1h \*\*\*Store this concentrated Stock at -20°C until ready to use\*\*\*

H20: 16 μl (This will be 1/20 dilution from Lucigen Stock)

Total: 20 μl

Note: Original Tn5 was diluted 10-fold (from 1 μl to 10 μl) after assembling, if 20-fold dilute needed, add more water.

For 1ng input DNA, use 10-fold diluted Tn5 transposome,

For 100pg or less, or single cell, use 20-fold diluted Tn5 transposome.

**Manual protocol:**

**3, Tagmentation and Neutralization**

DNA+H20: 4 μl

Assembled Tn5: 1 μl

2 X TD buffer: 5 μl

Total: 10 μl

55 °C for 10 min

NT buffer: 2.5 μl

25 °C for 6 min

**4, PCR**

KAPA HiFi Hotstart ReadyMix (2X): 25 μl

H2O: 10.5 μl

PCR-i5: 1~2μl

PCR-i7: 1~2μl

Total: 50 μl

72°C for 3 min, 98°C for 30s, (98°C for 10s, 63°C for 30s, 72°C for 1 min) 10-20 cycles.

1X-1.8X Ampure beads purify.

**Echo protocol:**

Such as single cell in 96 plate or 384 plates, please refer Haowei’s protocol but with the following modifications:

1, Sort cell or nuclei into the well before lysis buffer transfer.

2, For each PCR reaction:

2.5 μl: KAPA HiFi Hotstart ReadyMix (2X)

0.1 μl: PCR-i5 (10 μM)

0.1 μl: PCR-i7 (10 μM)

1.8 μl: H2O

Total: 4.5 μl

Using 384-pp\_plus-BP or 6RES-BP2