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TABLE-seq for strand-specific RNA sequencing

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We use this protocol and it's working

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Abstract

This protocol utilizes the Tn5, a widely used transposase, to perform single-strand DNA sequencing. Tn5 can tagment single-strand DNA and ligate transposon cargo to the 3′ end. We developed a tagmentation-based and ligation-enabled single-strand DNA sequencing method called TABLE-seq. The method below is an example to use TABLE-seq to conduct strand-specific RNA sequencing.

Troubleshooting

Before start

Assemble transposons and ligation adapters before starting the experiment.

Tn5 transposons Assembly

1. Add 1 µl 100 µM Tn5ME-B and 1 µl 100 µM Tn5MErev to 8 µl H_2O
2. Anneal oligos as Tn5-B at 95 °C 5 min, ramp to 25 °C at 5 °C/min, and hold at 16 °C.
3. Take 20 µl annealed Tn5-B and incubate with 70 µl of 200 µg/ml Tn5 transposase at 25 °C for 1 hour to assemble the single adaptor Tn5 transposon (Tn5B+B).
4. The assembled transposons can be stored at -20 °C until usage.

Tn5ME-B: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′
Tn5MErev: 5′- [phos]CTGTCTCTTATACACATCT-3′

Ligation adapter anneal

1. Add 2 µl 100 µM 5ph_Tn5a and 2 µl 100 µM Tn5a_N6_invert_dT to 16 µl H_2O
2. Anneal oligos as Adapter-A at 95 °C for 5 min, ramp to 25 °C at 5 °C/min, and hold at 16 °C.
3. Add 1 µl Exo I (NEB, Cat. # E1050), 3 µl 10X Exo I reaction buffer, and 6 µl H_2O
4. Incubate at 37 °C for 30 min, followed by 80 °C for 15 min, and 16 °C to hold.
5. Add 2X volume of VAHTS DNA clean beads (VAHTS, Cat. #N411-03), and gently mix by pipetting, place the tube at RT for 10 min.
6. Place the tube on the magnet stand for 1 min, and remove the liquid.
7. Add 200 µl 80% ethanol (fresh prepared) to the tube, keep the tube on the magnet stand, and do not mix or resuspend beads. Remove the ethanol after 30 s.
8. Repeat adding 200 µl 80% ethanol (fresh prepared) to the tube, keep the tube on the magnet stand, and do not mix or resuspend beads. Remove the ethanol after 30 s.
9. Air dry the beads for about 2-5 min. Do not over-dry the beads.
10. Remove samples for the magnet stand.
11. Resuspended beads with 20 µl H_2O, and incubate at RT for 5 min.
12. Place the tube on the magnet stand until the solution turns clear (around 30 s) and take all the liquid containing Adapter-A to the new tube.
13. Store at -20 °C until usage.

5ph_Tn5a: 5′- [phos]CTGTCTCTTATACACATCTGACGCTGCCGACGA-3′
Tn5a_N6_dT: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNN[invertdT] -3′

1 **Reverse transcription** 2h

Reverse transcription is conducted with Thermo Fisher SuperScript™ IV kit (Thermo, Cat. #18091050). Or any conventional method.

1.1 Take 300 ng (or any desired amount of) RNA, anneal with 1 µl 50 µM Oligo d(T)20 and 1 µl 10 mM dNTP at 65 °C for 5 min, and place on ice immediately for 2 min.
1.2 Mix each sample with 4 µl 5X SSIV Buffer, 1 µl 100 mM DTT, 1 µl Ribonuclease Inhibitor, 1 µl SuperScriptTM Reverse Transcriptase, 1 µl 100 µg/ml Actinomycin D, and add H2O to a total volume of 20 µl.
1.3 Incubate samples at 42 °C for 90 min, 10 cycles of 50 °C for 2 min and 42 °C for 2 min, then 85 °C for 5 min, and hold at 16 °C.
1.4 Add 1 µl Exo I (NEB, Cat. #M0293S), 3 µl 10X Exo I buffer, and 6 µl H2O to digest oligo d(T)20 primers at 37 °C for 30 min, then inactive Exo I at 80 °C for 15 min.
1.5 Add 1 µl RNase A (Takara, Cat. #2158) and 1 µl RNase H（Thermo, Cat. #18091050）and incubate at 37 °C for 30 min to digest the leftover RNA from cDNA.

2 **Tagmentation of cDNA or strand-specific RNA sequencing)** 40m

Single-strand DNA can be sequenced with the same TABLE-seq method.

1.1 Denature cDNA at 98 °C for 10 min and place samples on ice immediately for 5 min.
1.2 Mix 14 µl cDNA with 4 µl 5X DMF buffer (50% DMF, 50 mM Tris-HCl pH 7.5, 10 mM MgCl2) and 2 µl transposon Tn5 B+B.
1.3 Incubate samples at 37 °C for 5 min.
1.4. Stop the reaction by adding 2.4 µl Stop buffer (1.5 µl 0.5 M EDTA, 1.4 µl 10% SDS, and 0.5 µl 20 mg/ml Proteinase K) and incubating at 55 °C for 30 min and then 70 °C for 20 min.
1.5 Purify the tagmented cDNA by adding 0.9X volume of VAHTS DNA clean beads (VAHTS, Cat. #N411-03), and gently mixing by pipetting, place the tube at RT for 10 min.
1.6 Place the tube on the magnet stand for 1 min, and then remove the liquid.
1.7 Add 200 µl 80% ethanol (fresh prepared) to the tube, keep the tube on the magnet stand, and do not mix or resuspend beads. Remove the ethanol after 30 s.
1.8 Repeat adding 200 µl 80% ethanol (fresh prepared) to the tube, keep the tube on the magnet stand, and do not mix or resuspend beads. Remove the ethanol after 30 s.
1.9 Air dry the beads for about 2-5 min. Do not over-dry the beads.
1.10 Remove samples for the magnet stand.
1.11 Resuspended beads with 10 µl H2O, and incubate at RT for 5 min.
1.12 Place the tube on the magnet stand until the solution turns clear (around 30 s) and take all the liquid containing tagmented cDNA to the new tube.
1.13 Store at -20 °C until usage.

3 **RNA extractionRNA extraction is performed by RNeasy Plus Mini Kit (Qiagen, Cat. #74134). Or any conventional method.** 30m

1.1 Harvest and wash cells with PBS once.
1.2 Lyse the cell with 350 µl Buffer RLT and vortex for 30 s.
1.3 Add the lysates to a gDNA Eliminator spin column and centrifuge at 10,000 rpm for 30 sec.
1.4 Mix the flow-through with 350 µl of 70% ethanol before transferring the samples to an RNeasy spin column.
1.5 Centrifuge at 10,000 rpm for 15 s at RT.
1.6 Wash the RNA-bound column with 700 µl Buffer RW1, and centrifuge at 10,000 rpm for 15 s at RT.
1.7 Add 500 µl Buffer RPE to the RNA-bound column, and centrifuge at 10,000 rpm for 15 s at RT.
1.8 Repeat washing with 500 µl Buffer RPE once.
1.9 Place the column into a new 1.5 ml tube, add 30 µl of RNase-free H2O to the RNA-bound column, and centrifuge at 10,000 rpm for 15 s at RT to collect the RNA.
1.10 Treat 30 µl eluted RNA with 1 µl of DNase I (NEB, Cat. #M0303S), 5 ul 10X DNase I buffer, and 14 µl H2O, and incubate at 37 °C for 1 h to further eliminate genomic DNA.
1.11 Add 1 ml 75% ethanol to the treated RNA, and store at -20 °C for 1 hour.
1.12 Centrifuge samples at 12,000 rpm for 5 min at 4 °C and discard the supernatant.
1.13 Wash the precipitated RNA with 1 ml 75% ethanol twice.
1.14 Dissolved RNA with 30 µl RNase-free H2O.
1.15 Store the Purified RNA at -80 °C until usage.

4 Adapter ligation

1h 30m

1.1 Mix 10 µl tagmented cDNA with 2 µl annealed Adapter-A, 0.5 µl T4 DNA ligase (Takara, Cat. #2011A), 2 µl 10X ligation buffer, 2.5 µl 40% PEG6000, 3 µl H2O, and incubate at 37 °C for 1 h.
1.2 Purify the ligated cDNA by adding 0.8X volume of VAHTS DNA clean beads (VAHTS, Cat. #N411-03), and gently mixing by pipetting, place the tube at RT for 10 min.
1.3 Place the tube on the magnet stand for 1 min, and then remove the liquid.
1.4 Add 200 µl 80% ethanol (fresh prepared) to the tube, keep the tube on the magnet stand, and do not mix or resuspend beads. Remove the ethanol after 30 s.
1.5 Repeat adding 200 µl 80% ethanol (fresh prepared) to the tube, keep the tube on the magnet stand, and do not mix or resuspend beads. Remove the ethanol after 30 s.
1.6 Air dry the beads for about 2-5 min. Do not over-dry the beads.
1.7 Remove samples for the magnet stand.
1.8 Resuspended beads with 10 µl H2O, and incubate at RT for 5 min.
1.9 Place the tube on the magnet stand until the solution turns clear (around 30 s) and take all the liquid containing ligated cDNA to the new tube.
1.10 Store at -20 °C until usage.

5 Library construction

1h 30m

1.1 Amplify the purified DNA was amplified by primers with sequencing indexes.
1.2 Mix1 µl 10 µM Index-F primer, 1 µl 10 µM Index-R primer, 8 µl ligated DNA, and 10 µl 2X HIFI PCR Master Mix (NEB, Cat. #M0541) and incubate at 72 °C for 5 min, 98 °C for 30 s, 20 cycles of 98 °C for 10 s and 63 °C for 10 s, then 72 °C for 1 min, and hold at 16 °C.
1.3 Purified PCR products by adding 0.9X volume of VAHTS DNA clean beads (VAHTS, Cat. #N411-03), and gently mixing by pipetting, place the tube at RT for 10 min.
1.4 Place the tube on the magnet stand for 1 min, and then remove the liquid.
1.5 Add 200 µl 80% ethanol (fresh prepared) to the tube, keep the tube on the magnet stand, and do not mix or resuspend beads. Remove the ethanol after 30 s.
1.6 Repeat adding 200 µl 80% ethanol (fresh prepared) to the tube, keep the tube on the magnet stand, and do not mix or resuspend beads. Remove the ethanol after 30 s.
1.7 Air dry the beads for about 2-5 min. Do not over-dry the beads.
1.8 Remove samples for the magnet stand.
1.9 Resuspended beads with 20 µl H2O, and incubate at RT for 5 min.
1.10 Place the tube on the magnet stand until the solution turns clear (around 30 s) and take all the liquid containing constructed libraries to the new tube.
1.11 Detect concentrations of the constructed libraries by adding 1 µl DNA to 199 µl Equalbit 1X dsDNA HS Working Solution (Vazyme, Cat. #121-01-AA). Or any preferred method.
1.12 Library sequencing is performed with an Illumina NovaSeq platform with pair-end reads of 150 bp. Or any preferred method.

Index-F primer: AATGATACGGCGACCACCGAGATCTACAC XXXXXXXX TCGTCGGCAGCGTCAGATGTGTAT (XXXXXXXX is the index sequence for sequencing)
Index-R primer: CAAGCAGAAGACGGCATACGAGAT XXXXXXXX GTCTCGTGGGCTCGGAGATGTG (XXXXXXXX is the index sequence for sequencing)