Stacc-seq protocol (version 5)

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Stacc-seq: **S**mall-scale **T**n5-**a**ssisted **C**hromatin **C**leavage with **seq**uencing

## Reagents

- Ethanol
- dd$H_2O$
- pA/G-Tn5 (Tn5 and proteinA/G fusion protein, Vazyme TD901, TD902)
- AMPure beads
- Concanavalin-coated magnetic beads (Polyscience, 86057)
- Qubit DNA HS
- Phenol-chloroform
- Carrier RNA (50x dilute first to make 20ng/ul solution)
- NaOAc (3M)
- Glycogen
- 5x TTBL (Vazyme, TD502)
- PCR kit for Tn5 (Vazyme, TD601)
- Digitonin (Thermo, BN2006)
- PCR primer for Tn5 (Vazyme, TD202)
- TE buffer (Tris-EDTA, for DNA)

- Buffer1

| | |
|---|---|
| Tris-HCl (pH 7.4) | 10mM |
| NaCl | 150mM |
| Spermidine | 0.5mM |
| Roche complete (EDTA free) | 1X |

- Buffer2

| | |
|---|---|
| Tris-HCl (pH 7.4) | 10mM |
| KCl | 10mM |
| $CaCl_2$ | 1mM |
| $MnCl_2$ | 1mM |

## Materials

- Magnetic rack
- PCR thermal cycler
- Incubator
- Thermo mixer
- Low binding EP, PCR tube and pipette tube
- 4°C Centrifuge
- Phase-lock tube

## Procedures

1. Harvest and count cells.

- Cells should be harvested freshly and frozen pellet cells do not guarantee good quality of Stacc-seq library
- Cells should be intact and in a homogenous, single-cell suspension.

2. Antibody and pA/G-Tn5 complex preparation

- Prepare D-buffer 1 (DB-1): add 1ul fully dissolved 5% digitonin (@95° C, >5min) into 1ml Buffer1 and vortex.
- Add 7ul DB-1, 0.5ul pA/G-Tn5, 0.5**ug** antibody into 200ul low-binding tube and vortex.
- Incubate at 4°C for 30min.

**Stacc-seq without wash step:**

3-1. Prepare cell lysis

- Add 1M~500 cells into 200ul low-binding tube (volumn < 1ul).
- Add 6ul DB-1 into cells and vortex.
- Incubate at 4°C for 10min, vortex each 2.5min for 3 times.

4-1. ChIC via anbibody-Tn5 complex

- Add 35ul DB-1 into cell lysis, then add antibody and pA/G-Tn5 mixture.
- Add 12.5ul pre-warmed 5xTTBL.
- Incubate in Thermo mixer at 37°C for 30min (400rpm, 10s ON, 10s OFF)

**Stacc-seq with wash step:**

3-2. Prepare cell lysis

- Add 1M~500 cells into 200ul low-binding tube (volumn < 1ul).

- Suspend cell with 50ul Buffer 1.
- Add 10ul pre-washed ConA beads (wash with 200ul Buffer 2 once and resuspend in 10ul Buffer 2), and incubate at room temperature for 10min.
- Remove supernatant on a magnet stand, and then washed with 200ul Buffer 1 twice.
- Remove supernatant on a magnet stand, and then add pA/G–Tn5 mixture and DB-1 buffer (50 μl in total)
- Incubate on an Eppendorf Thermomixer at 4 °C, 400 rpm for 2 h.
- Remove supernatant on a magnet stand, and wash twice with 200ul DB-1 buffer.
- Suspend beads with 50ul DB-1 buffer.
- Add 12.5ul 5xTTBL.

4-2. ChIC via anbibody-Tn5 complex

- Suspend beads with 50ul DB-1 buffer.
- Add 12.5ul pre-warmed 5xTTBL.
- Incubate in Thermo mixer at 37°C for 30min (400rpm, 10s ON, 10s OFF)

**Follow step 4-1 or 4-2:**

5. Phenol-chloroform Purification

- Add 2ul carrier RNA, 2ul spike-in DNA (optional) and 65ul TE into cell lysis.
- Transfer the mixture into a phase-lock tube.
- Add 130ul Phenol-chloroform and vortex for 15s.
- Incubate at room temperature for 5min.

- 12,000 rpm centrifuge 5 min and extract supernatant
- Add 650ul ethanol, 24ul NaOAc, 2ul glycogen
- Incubate at -20°C 30min or overnight (for low input cells).
- Spin at 4°C for 15 min at maximum speed.
- Remove supernatant and wash pellets with 75% ethanol. Air dry and resuspend pellets in 29ul H2O.

6. PCR reaction

make the 50 μL PCR reaction mix in a 0.2 mL PCR tube to amplify transposed DNA fragments:

- Be careful to ensure that samples are barcoded appropriately for subsequent pooling and sequencing.
- The first 3min extension at 72 °C is critical to allow extension of both ends of the primer after transposition, thereby generating amplifiable fragments.

| | |
|---|---|
| DNA product | 29ul |
| 5xTAB | 10ul |
| N5XX | 5ul |
| N7XX | 5ul |
| TAE | 1ul |

Thermal cycle as follows:

| | | |
|---|---|---|
| 72°C | | 3min |
| 98°C | | 30sec |
| 16cycle | 98°C | 15sec |

| | | |
|---|---|---|
| | 60°C | 30sec |
| | 72°C | 3min |
| 72°C | | 5min |
| 4°C | | hold |

7. Purify amplified library using AMPure Beads.
    - 0.4x beads were used to filter out large fragments
    - 1.7x beads were used to filter out small fragments
    - Elute the purified library in 20 µL dd$H_2O$

8. Library QC and quantification
    - Measure the concentration of libraries by using Qubit.
    - Quantify relative amount of libraries by using qPCR Illumina protocol.
    - Pool your libraries according to qPCR results above.