mini ATAC-seq protocol

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Reagents

- Ethanol
- ddH_2O
- Lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40 or 0.005% digitonin)
- TruePrep DNA Library Prep Kit V2 for Illumina (TD502 96 rxn, Vazyme)
- AMPure Beads
- Qubit DNA HS
- Phenol-chloroform (Amresco K169, pH 8.0)
- Carrier RNA (50x dilute first to make 20ng/ul solution, EpiTect Fast DNA Bisulfite Kit 59824)
- NaOAc (3M)
- Glycogen
- Tris-EDTA (pH7.5)

Materials

- Magnetic rack
- PCR thermal cycler
- Incubator
- Low binding EP, PCR tube and pipette tips
- 4^oC Centrifuge
- phase-lock tube (WM5-2302820 TIANGEN)

Procedures

1. Harvest and count cells

Cells should be harvested freshly and frozen pellet cells do not guarantee good quality of ATAC-seq library. 20-5,000 cells are recommended.

Cells should be intact and in a homogenous, single-cell suspension

2. Nuclei preparation.

Be careful since cell pellet would be hard to see by eye. Make sure the cell pellet is placed on ice.

- Centrifuge cells in 200ul tube for 5 min at 500g, 4oC
- Remove and discard the supernatant under the stereomicroscope or microscope

3. Lysis

- 6ul lysis buffer were added to the sample to resuspend. To avoid mtDNA contamination, add 1ul 5% pre-warmed (95°C, 5min) digitonin to 1ml no-detergent lysis buffer. Digitonin lysis buffer must be freshly used.
- Incubate on ice for 10 min

4.Transposition/Tagmentation

Tagmentation reagents was added to the sample without lysis removal.

ddH_2O	5ul
5xTTBL (Tagmentation buffer)	4ul
TTE mix V5 (Tn5 Transposase)	5ul
Nuclei pellet	6ul

- Mix and incubate the transposition reaction at 37 ^oC for 30 min

4. STOP tagmentation

This step is to strip Tn5 from the DNA to facilitate the PCR reaction.

- add 5ul 5xTS buffer, mix and incubate at the room temperature for 5min

5. Phenol-chloroform Purification

- Prepare the extraction mix，vortex to mix and stand at room temperature for 3 min

DNA	25ul
Carrier RNA (20ng/ul)	2ul

TE (Tris-EDTA)	73ul
Phenol-chloroform	100ul

- Transfer to phase-lock tube and 12,000 rpm centrifuge 5 min and extract supernatant
- Supernatant was transferred to a new 1.5ml tube for ethanol precipitation

DNA	~100ul
Ethanol	650ul
NaOAc	24ul
Glycogen	2ul

- Incubate at -20C overnight.
- In the next day, spin at 4^{o}C for 15 min at maximum speed
- Remove supernatant and wash pellets with 75% ethanol. Air dry and resuspend pellets in 29ul ddH_2O

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 ^{o}C is critical to allow extension of both ends of the primer after transposition, thereby generating amplifiable fragments.

- Make a PCR mix:

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

Thermal cycle as follows:

72°C		3min
98°C		30sec
	98°C	15sec
16~18 cycle	60°C	30sec
	72°C	3min
72°C		5min
4°C		hold

6. Purify amplified library using AMPure Beads.

- 0.5x-1.5x beads were used for size selection.
- Elute the purified library in 20 μL ddH_2O.

7. Library QC and quantification

- Measure the concentration of libraries by using Qubit.

REFERENCES

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