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ATAC-seq library preparation in cultured cells

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We use this protocol and it's working

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Disclaimer

This protocol is provided for research use only and may require optimization depending on experimental conditions.

Abstract

This protocol describes assay for transposase-accessible chromatin using sequencing (ATAC-seq) in cultured cells, including nuclei preparation, transposition reaction, DNA purification, and library amplification.

Materials

- ATAC-Resuspension buffer (ATAC-RSB)**: 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, supplemented with 0.1% NP-40, 0.1% Tween-20, 0.01% digitonin
- Transposition master mix**: 2x TD buffer (20 mM Tris-HCl, pH 7.6; 10 mM $MgCl_2$; 20% dimethylformamide)
- Tn5 transposase (Illumina, 20034197),
- 1% digitonin (Millipore, 300410),
- 10% Tween-20 (Sigma/Roche cat# 11332465001)
- MinElute PCR purification kit (QIAGEN, 28004)
- 1x NEBNext PCR Master Mix**

Safety warnings

- Cell number and lysis conditions may require optimization depending on cell type.
- Over-lysis can reduce library complexity.
- Accurate pipetting during transposition is critical for reproducibility.

Before start

- Perform all steps on ice or at 4°C unless otherwise indicated.
- Use freshly prepared buffers or thawed aliquots kept on ice.
- Count viable cells accurately prior to starting the assay.
- Pre-chill centrifuges and rotators.
- Prepare transposition master mix immediately before use.

Cell preparation and lysis

3m

1 Collect 50,000 viable cells per sample.

2 Resuspend cells in 50 µL cold ATAC-Resuspension buffer (ATAC-RSB) containing:
10 mM Tris-HCl (pH 7.4)
10 mM NaCl
3 mM $MgCl_2$

Supplemented with:
0.1% NP-40
0.1% Tween-20
0.01% digitonin

3 Incubate cell suspension on ice for 3 min. 3m

4 Wash the lysate with 1 mL of cold ATAC-RSB without NP-40 and digitonin.

5 Pellet nuclei by centrifugation at 500 xg and carefully remove supernatant.

Transposition reaction

30m

6 Resuspend nuclei in 50 µL transposition master mix containing:
2x TD buffer (20 mM Tris-HCl, pH 7.6; 10 mM $MgCl_2$; 20% dimethylformamide)
2.5 µL Tn5 transposase (Illumina, 20034197)
16.5 µL PBS
0.5 µL 1% digitonin (Millipore, 300410)
0.5 µL 10% Tween-20

7 Incubate transposition reaction at 37°C for 30 min with 1,000 rpm mixing. 30m

DNA purification

30m

8 Purify transposed DNA fragments using the MinElute PCR purification kit (QIAGEN, 28004) according to the manufacturer's instructions. 30m

9 Elute purified DNA in 20 µL nuclease-free water or elution buffer.

Library amplification 2h

10 Construct sequencing libraries using 1x NEBNext PCR Master Mix and custom primers as described by Buenrostro et al. 1h

11 Amplify libraries according to standard ATAC-seq PCR conditions. 1h

Protocol references

Corces, M. R. et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat. Methods 14, 959–962 (2017).

Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. & Greenleaf, W. J. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat. Methods 10, 1213–1218 (2013).