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Omni-ATAC

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We use this protocol and it's working

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Disclaimer

This protocol was adapted from the work of Dahlia Rohm and colleagues in the Gersbach lab at Duke University.

Abstract

This protocols describes methods for ATAC-seq in human induced pluripotent stem cells.

Troubleshooting

Cell Preparation

1 Harvest cells (no fixation), protocol to be defined by the user. Spin down cells at 500 ×g for 5 min, 4°C. Keep cells on ice for all steps. Ideally, do not process more than 6-8 samples at a time. Cells should be processed fresh.

Frozen cells were not used for this protocol, although frozen cells may be used.

2 Aspirate all supernatant, carefully avoiding cell pellet (may not be visible at this stage), using two pipetting steps (aspirate down to 20-30ul with a p1000 pipette and remove final volume with a p10).

3 Add 50 µL of cold RSB (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2) containing 0.1% NP40, 0.1% Tween-20 and 0.01% Digitonin. Using wide bore tips gently pipette 3 times to suspend the cell pellet.

4 Incubate on ice for 3 minutes.

5 Wash out lysis with 1mL of cold RSB containing 0.1% Tween-20 and invert tube 3 times to mix

6 Spin down at 500 ×g for 10 min, 4°C.

7 Aspirate all supernatant, carefully avoiding visible cell pellet, using two pipetting steps. Suspend nuclei pellet in 27ul 2X TD Buffer by pipetting up and down 5 times. Use 2ul to count nuclei. If nuclei are too concentrated to accurately count, dilute further with 2X TD Buffer. Adjust 50,000 nuclei to 25ul total using 2X TD Buffer.

Transposition Reaction and Purification

8 Prior to transposition: make sure cells are viable.
For samples with more than 15-20% dead cells, discard or separate viable cells over Ficoll.

9 Make sure the cell pellet is set on ice.

10 To make the transposition reaction mix, combine the following (prepare a master mix) then add 25ul to each sample:
2.5 µL Tn5 Transposase (#200341970)
16.5 µL 1X PBS

0.5 µL 10% Tween-20
0.5 µL 1% Digitonin (Invitrogen #BN2006)
5 µL Nuclease Free H2O
50 µl Total reaction volume

11 Gently pipette 3 times to mix nuclei suspension with the transposition reaction mix.

12 Incubate the transposition reaction at 37°C for 30 min in a thermomixer with 1000 RPM mixing.

13 Immediately following transposition, purify using a Zymo DNA Clean up Kit (#11-303C).

14 Elute transposed DNA in 11 µL Elution Buffer (10mM Tris buffer, pH 8).

15 Purified DNA can be stored at -20°C.

PCR Amplification

16 To amplify transposed DNA fragments, combine the following in a PCR tube:
10 µL Transposed DNA (all)
2.5 µL 25µM Customized Nextera PCR Primer 1*
2.5 µL 25µM Customized Nextera PCR Primer 2*
1 µL KAPA HiFi polymerase (KK2101)
1.5 µL dNTPs (10mM stock)
10 µL 5x KAPA HF buffer
22.5 µL Nuclease free H2O
50 µL Total
*Complete list of primers available in Indexes section of this protocol

17 Cycle as follows for the pre-amplification reaction:
(1) 72°C, 5 min
(2) 98°C, 30 sec
(3) 98°C, 10 sec
(4) 65°C, 30 sec
(5) 72°C, 1 min
(6) Repeat steps 3-5, 4x (5 cycles total)
(7) Hold at 4°C

18 Remove tubes from thermocycler and store on ice. Proceed to qPCR amplification to determine additional cycles immediately. To run a qPCR side reaction, combine the following:
5 µL PCR pre-amplified DNA (10% of initial reaction)
5.26 µL Nuclease Free H2O
0.45 µL 25µM Customized Nextera PCR Primer 1
0.45 µL 25µM Customized Nextera PCR Primer 2 (Barcode)
0.09 µL 100x SYBR Green I* (Invitrogen Cat #S-7563)
0.45 µL dNTPs (10mM stock)
0.3 µL KAPA HiFi polymerase (KK2101)

15 µL Total

*10,000x SYBR Green I is diluted in 10mM Tris buffer, pH 8 to make a 100x working solution

19 qPCR cycle as follows:
(1) 98°C, 30 sec
(2) 98°C, 10 sec
(3) 65°C, 30 sec
(4) 72°C, 1 min
(5) Repeat steps 2-4, 19x
(6) Hold at 4°C

20 After qPCR amplification, manually assess the amplification profiles and determine number of additional cycles to amplify. See Buenrostro et al 2015 (PMID: 25559105) for a detailed explanation. Basically, the additional number of cycles needed for the remaining 45 µL PCR reaction is determined as following:
(1) Plot linear Rn vs. Cycle
(2) Calculate the # of cycle that corresponds to ¼ of maximum fluorescent intensity

21 Using the remaining 45 µL PCR reaction, run the required number of additional cycles. Most libraries will need 4-8 additional cycles. Anything needing more than 15 additional cycles should be considered as failed. Place the pre-amplified tubes back in the thermocycler without addition of any more reagents. Cycle as follows:
(1) 98°C, 30 sec
(2) 98°C, 10 sec
(3) 65°C, 30 sec
(4) 72°C, 1 min
(5) Repeat steps 2-4, n_k-1 times for n_k cycles total (where n_k is the number of additional cycles needed as determined in step 6 for sample k)
(6) Hold at 4°C

22 Purify amplified library using 2X ratio of Ampure XP beads. Elute the purified library in 20 µL Elution Buffer (10mM Tris Buffer, pH 8).

23 The workflow for the PCR purification process is as follows:
1. Add 2 µL AMPure beads per 1.0 µL of sample (50µl for a 45µl PCR reaction).
2. Transfer to Eppendorf microcentrifuge tube and mix 10 times. Incubate 10min at RT to bind DNA fragments to paramagnetic beads.
3. Add magnet and wait until solution clears, about 5 min.
4. Wash beads + DNA fragments twice with fresh 80% Ethanol to remove contaminants. With beads on magnet, add 200µl EtOH (or enough to cover the beads), leave 30 seconds, remove all EtOH. Repeat once. Air dry beads 2-3min at RT, no more than 5 min.
5. Elute purified DNA fragments from beads. Remove magnet and suspend beads with 20 µl of 10mM Tris, pH 8. Mix10 times. Incubate 5 min then separate with magnet.
6. Transfer to new tube.

24 Use Agilent TapeStation (or similar device) to run a D5000 tape and check quality of purified DNA fragments (nucleosomal banding pattern should be present). Identify the average fragment size for each sample.

25 Use a Qubit dsDNA BR Assay Kit (Invitrogen cat no. Q32850, Q32853) and a Qubit Fluorometer to measure concentration of each sample.

26 Use average fragment size and Qubit concentration to calculate the molarity of each sample. Make an equimolar pool of all samples for sequencing, and follow sample preparation and sequencing instructions for Illumina NextSeq 2000 or NovaSeq sequencing systems for 2×25bp paired-end reads.

Indexes

27 A full list of Illumina adapters can be found at
Illumina Adapter Sequences (1000000002694)

Nextera DNA indexes (page 16-17)

28 N701: CAAGCAGAAGACGGCATACGAGAT**TCGCCTTA**GTCTCGTGGGCTCGGAGATGT

N702: CAAGCAGAAGACGGCATACGAGAT**CTAGTACG**GTCTCGTGGGCTCGGAGATGT

N703: CAAGCAGAAGACGGCATACGAGAT**TTCTGCCT**GTCTCGTGGGCTCGGAGATGT

N704: CAAGCAGAAGACGGCATACGAGAT**GCTCAGGA**GTCTCGTGGGCTCGGAGATGT

N705: CAAGCAGAAGACGGCATACGAGAT**AGGAGTCC**GTCTCGTGGGCTCGGAGATGT

N706: CAAGCAGAAGACGGCATACGAGAT**CATGCCTA**GTCTCGTGGGCTCGGAGATGT

N707: CAAGCAGAAGACGGCATACGAGAT**GTAGAGAG**GTCTCGTGGGCTCGGAGATGT

N708: CAAGCAGAAGACGGCATACGAGAT**CCTCTCTG**GTCTCGTGGGCTCGGAGATGT

29 N501:
AATGATACGGCGACCACCGAGATCTACAC*TAGATCGC*TCGTCGGCAGCGTCAGATGTG

N502:
AATGATACGGCGACCACCGAGATCTACAC*CTCTCTAT*TCGTCGGCAGCGTCAGATGTG

N503:
AATGATACGGCGACCACCGAGATCTACAC*TATCCTCT*TCGTCGGCAGCGTCAGATGTG

N504:
AATGATACGGCGACCACCGAGATCTACAC*AGAGTAGA*TCGTCGGCAGCGTCAGATGTG

N505:
AATGATACGGCGACCACCGAGATCTACAC*GTAAGGAG*TCGTCGGCAGCGTCAGATGTG

N506:
AATGATACGGCGACCACCGAGATCTACAC*ACTGCATA*TCGTCGGCAGCGTCAGATGTG

N507:
AATGATACGGCGACCACCGAGATCTACAC*AAGGAGTA*TCGTCGGCAGCGTCAGATGTG

N508:
AATGATACGGCGACCACCGAGATCTACAC*CTAAGCCT*TCGTCGGCAGCGTCAGATGTG

Protocol references

Protocol adapted from:
Corces MR, Trevino AE, Hamilton EG, Greenside PG, Sinnott-Armstrong NA, Vesuna S, Satpathy AT, Rubin AJ, Montine KS, Wu B, Kathiria A, Cho SW, Mumbach MR, Carter AC, Kasowski M, Orloff LA, Risca VI, Kundaje A, Khavari PA, Montine TJ, Greenleaf WJ, Chang HY. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat Methods. 2017 Oct;14(10):959-962. doi: 10.1038/nmeth.4396. Epub 2017 Aug 28. PMID: 28846090; PMCID: PMC5623106.