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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 Corces et al. *Nat Methods*, 2017 for use in the Gersbach lab.

Abstract

This protocol describes methods for performing ATACseq.

Materials

DNase (Worthington #LS002007), HBSS, PBS, Ficoll, RSB (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$), NP40, Tween-20, Digitonin, 2X TD Buffer (20mM Tris-HCl pH7.6, 10mM $MgCl_2$, 20% Dimethyl Formamide), Tn5 Transposase (#200341970), Nuclease Free H_2O, Zymo DNA Clean up Kit (#11-303C), Elution Buffer (10mM Tris buffer, pH 8), Customized Nextera PCR Primer 1, Customized Nextera PCR Primer 2, NEBNext High-Fidelity 2x PCR Master Mix (New England Labs Cat #M0541), SYBR Green I (Invitrogen Cat #S-7563), AMPure XP beads, Illumina Adapter Sequences, Nextera DNA indexes.

Troubleshooting

Before start

Prior to transposition: make sure cells are viable.

For samples with 5-15% dead cells, treat cells in culture with DNase (Worthington #LS002007) at a final concentration of 200 U/mL. Suspend DNase in HBSS. Treat for 30 minutes at 37C. Wash thoroughly with PBS to remove DNase prior to proceeding to ATAC-seq.

For samples with more than 15-20% dead cells, separate viable cells over Ficoll.

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 can be viably frozen to be processed later. If using frozen cells, quickly thaw cells at 37°C and suspend with 10mL growth media. Centrifuge 5 min at 1000 rpm. Wash with 1mL cold PBS once, spin down and proceed to step 2.

2 Aspirate all supernatant, carefully avoiding cell pellet (may not be visible at this stage), using two pipetting steps (aspirate down to 20-30 µL 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 27 µL 2X TD Buffer by pipetting up and down 5 times. Use 2 µL to count nuclei. If nuclei are too concentrated to accurately count, dilute further with 2X TD Buffer. Adjust 50,000 nuclei to 25 µL total using 2X TD Buffer.

Transposition Reaction and Purification

8 Make sure the cell pellet is set on ice.

9 To make the transposition reaction mix, combine the following (prepare a master mix) then add 25 µL 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

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

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

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

13 Elute transposed DNA in 21 µL Elution Buffer (10mM Tris buffer, pH 8).

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

PCR Amplification

15 To amplify transposed DNA fragments, combine the following in a PCR tube:
20 µL Transposed DNA
2.5 µL 25µM Customized Nextera PCR Primer 1∗
2.5 µL 25µM Customized Nextera PCR Primer 2∗
25 µL NEBNext High-Fidelity 2x PCR Master Mix (New England Labs Cat #M0541)
50 µL Total
∗Complete list of primers available in Section VI of this protocol

16 Cycle as follows:
(1) 72°C, 5 min
(2) 98°C, 30 sec
(3) 98°C, 10 sec
(4) 63°C, 30 sec
(5) 72°C, 1 min
(6) Repeat steps 3-5, 4x
(7) Hold at 4°C

17 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 5 cycles PCR amplified DNA
4 µL Nuclease Free H2O
0.5 µL 50µM Customized Nextera PCR Primer 1
0.5 µL 50µM Customized Nextera PCR Primer 2 (Barcode)

0.06 µL 100x SYBR Green I∗ (Invitrogen Cat #S-7563)
10 µL NEBNext High-Fidelity 2x PCR Master Mix
20 µL Total

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

18 qPCR cycle as follows:
(1) 98°C, 30 sec
(2) 98°C, 10 sec
(3) 63°C, 30 sec
(4) 72°C, 1 min
(5) Repeat steps 2-4, 19x
(6) Hold at 4°C

19 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:

19.1 (1) Plot linear Rn vs. Cycle

19.2 (2) Calculate the # of cycle that corresponds to ¼ of maximum fluorescent intensity

20 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) 63°C, 30 sec
(4) 72°C, 1 min
(5) Repeat steps 2-4, x times
(6) Hold at 4°C

21 Purify amplified library using 1.2X ratio of Ampure XP beads. Elute the purified library in 20 µL Elution Buffer (10mM Tris Buffer, pH 8).

22 The workflow for the PCR purification process is as follows:

22.1 Add 1.2 µL AMPure beads per 1.0 µL of sample (50 µL for a 45 µL PCR reaction).

22.2 Transfer to Eppendorf tube and mix 10 times. Incubate 10 min at RT to bind DNA fragments to paramagnetic beads.

22.3 Add magnet and wait until solution clears, about 5 min.

22.4 Wash beads + DNA fragments twice with fresh 80% Ethanol to remove contaminants. With beads on magnet, add 200 µL EtOH (or enough to cover the beads), leave 30 seconds, remove all EtOH. Repeat once. Air dry beads 2-3 min at RT, no more than 5 min.

22.5 Elute purified DNA fragments from beads. Remove magnet and suspend beads with 20 µL of 10mM Tris, pH 8. Mix 10 times. Incubate 5 min then separate with magnet.

22.6 Transfer to new tube.

Indexes

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

24 Nextera DNA indexes (page 16-17):
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

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

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