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Bulk ATAC-seq

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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 Boxun Li and Alexis Safi in the Gersbach lab at Duke University.

Abstract

This protocol describes methods for performing bulk ATAC-seq on iPSC-derived neurons and mouse primary astrocytes.

Materials

KnockOut Serum Replacement (Gibco, 10828010)
dimethyl sulfoxide (Sigma-Aldrich, D8418)
Zymo DNA Clean up Kit (#11-303C)
NEBNext High-Fidelity 2x PCR Master Mix (New England Labs Cat #M0541)
100x SYBR Green I* (Invitrogen Cat #S-7563)

Cell Preparation

1 Grow and dissociate neuron and astrocyte monocultures and neuron-astrocyte co-cultures (according to previous protocols Ngn2 neuron differentiation [Ref. 2], and 3.Neuron dissociation [Ref. 3]) to neuronal maturation Day 3, 7, 14, and 28 (i.e., co-culture Day 2, 6, 13, and 27). Viably freeze dissociated single cells in 90% KnockOut Serum Replacement (Gibco, 10828010) + 10% dimethyl sulfoxide (Sigma-Aldrich, D8418).

2 Upon completion of sample collection, thaw frozen vials from various time points together at 37C. Use approximately 100,000-500,000 cells for ATAC-seq library preparation. Cells were washed with warm Neuronal Maturation Medium (detailed in protocol Ngn2 neuron differentiation [Ref.2]). Centrifuge cells for 5 mins at 1000 rpm. Wash with 1mL cold 1X PBS. Centrifuge again for 5 mins at 1000 rpm.

3 Resuspend pellet with 1mL of cold ATAC-seq resuspension buffer (RSB; 10 mM Tris-HCl pH 7.4, 10 mM NaCl, and 3 mM $MgCl_2$ in water) + 0.1% Tween. Transfer to 1mL dounce homogenizer.

4 Give 10 stokes with tight pestle. Transfer to Eppendorf tube.

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

6 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 (10ul trypan blue+2ul nuclei suspension+8ul water). 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.

[2X TD Buffer: 20mM Tris-HCl pH7.6, 10mM MgCl2, 20% Dimethyl Formamide]

Transposition Reaction and Purification

7 Make sure the cell pellet is set on ice.

8 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

9 Using wide bore tip, gently pipette 3 times to mix nuclei suspension with the transposition reaction mix.

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

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

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

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

PCR Amplification

14 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 Indexes of this protocol

15 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

16 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

17 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

18 After qPCR amplification, manually assess the amplification profiles and determine number of additional cycles to amplify. See Buenrostro et al 2015 (Ref. 4) for a detailed explanation. 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

19 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

20 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).

21 The workflow for the PCR purification process is as follows:
1. Add 1.2 µL AMPure beads per 1.0 µL of sample (50µl for a 45µl PCR reaction).
2. Transfer to eppendorf 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.

Indexes

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

Nextera DNA indexes (page 16-17)

23 N701: CAAGCAGAAGACGGCATACGAGAT**TCGCCTTA**GTCTCGTGGGCTCGGAGATGT

N702: CAAGCAGAAGACGGCATACGAGAT**CTAGTACGG**TCTCGTGGGCTCGGAGATGT

N703: CAAGCAGAAGACGGCATACGAGAT**TTCTGCCT**GTCTCGTGGGCTCGGAGATGT

N704: CAAGCAGAAGACGGCATACGAGAT**GCTCAGGA**GTCTCGTGGGCTCGGAGATGT

N705: CAAGCAGAAGACGGCATACGAGAT**AGGAGTCC**GTCTCGTGGGCTCGGAGATGT

N706: CAAGCAGAAGACGGCATACGAGAT**CATGCCTA**GTCTCGTGGGCTCGGAGATGT

N707: CAAGCAGAAGACGGCATACGAGAT**GTAGAGAG**GTCTCGTGGGCTCGGAGATGT

N708: CAAGCAGAAGACGGCATACGAGAT**CCTCTCTG**GTCTCGTGGGCTCGGAGATGT

24 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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4. Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. **ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide.** Curr Protoc Mol Biol. 2015. PMID: 25559105

Acknowledgements

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