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Based on protocol from Dr Stephen Parker lab (UMICH)

ATAC-Seq on Liver nuclei

Materials

Isolated nuclei frozen in Nuclei storage buffer
Ice-cold PBS
Refrigerated 5430R centrifuge
Non-refrigerated centrifuge
Nextera DNA Library Preparation kit (Illumina cat. FC-121-1030)
Qiagen MinElute PCR Purification kit
100x SYBR Green I
NEBNext High-Fidelity 2x PCR Master Mix (NEB cat no. M0541)

Procedure

1. Take aliquot of nuclei so that you have 50,000 in an eppendorf
2. Bring volume up to 50ul with cold PBS buffer. Centrifuge 5 min at 500 xg, 4C
3. Remove and discard supernatant, make sure the nuclei pellet is on ice.
4. Immediately continue to transposition reaction
5. Make the transposition reaction mix, combining the following:

Table1

	A	B	C	D
1	Transposition Mix	1x	5x	
2	TD (2× reaction buffer from Nextera kit)	25	125	µl
3	nuclease-free H2O	22.5	112.5	µl

6. Resuspend the nuclei pellet in the transposition reaction mix
7. Add 2.5ul TDE1 (Nextera Tn5 Transposase) to each sample
8. Incubate the transposition reaction at 37C for 30 minute on the thermomixer, with gentle mixing (300 rpm)
9. Immediately following transposition, purify using a Qiagen MinEllute PCR purification kit
10. Elute transposed DNA in 10ul of EB

PCR amplification 1

11. Combine the following reagents in a 1.5ml PCR tube

Table2

	A	B	C	D
1	PCR 1 Mix	1x	9x	
2	transposed DNA	10	-	µl
3	nuclease-free H2O	10	90	µl
4	25 µM Ad1_noMX FWD PRIMER	2.5	22.5	µl
5	NEBNext High-Fidelity 2× PCR Master Mix	25	225	µl

12. Add 37.5ul of the master mix to each 0.2 ml PCR tube, 2.5ul of the unique barcoded reverse primer, and 10ul purified transposed DNA.
13. Run the following PCR program (ATAC-Seq PCR 1)

Table3

	A	B
1	1 cycle:	5 min 72°C
2		30 sec 98°C
3	5 cycles:	10 sec 98°C
4		30 sec 63°C
5		1 min 72°C.

14. the first 5-min extension at 72C is critical to allow extension of both ends of the primer after transposition, thereby generating amplifiable fragments. This short pre-amplification step ensures that downstream quantitative PCR (qPCR) will not change the complexity of the original library
15. If not continuing to the following step, the samples can be put at -20 until further qPCR quantitation and final library amplification. If continuing with the qPCR to choose the additional number of cycles required for library amplification, the PCR reactions can be stored at 4C until the qPCR is done

qPCR to choose cycle number

16. Make the following master mix

Components	1x	9x
SYBR Green 100x	0.09ul	0.81ul
Nuclease-free H2O	4.41ul	39.69ul
NEBNext High-Fidelity 2x PCR master mix	5ul	45ul
Ad1_noMX FWD primer	0.25ul	2.25ul

17. add 9.75ul of the master mix to a 0.2ml PCR tube
18. Add 0.25ul of the Reverse primer
19. Add 5ul of template (coming from PCR 1)
20. Run the following cycles on the StepOnePlus qPCR machine

Table5

	A	B
1	1 cycle:	30 sec 98°C
2		
3	30 cycles:	10 sec 98°C
4		30 sec 63°C
5		1 min 72°C.

21. Export the cycling data to an excel spreadsheet, and calculate the number of cycles required for 1/3 of plateau'ed amplification

22. Run the remaining 45ul of initial (PCR1) reaction, adjusting the cycle number as needed for each sample
23. After PCR, use the MinElute for final cleanup. Elute in 35ul Elution buffer
24. Quantify using Qubit HS dsDNA kit, and run on HS D1000 TapeStation kit

Primers:

Table6

	A	B
1	Ad1_noMX	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
2	Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
3	Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
4	Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
5	Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
6	Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
7	Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
8	Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
9	Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
10		
11	Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
12	Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
13	Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
14	Ad2.12_GTAGAGGA	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
15	Ad2.13_GTCGTGAT	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
16	Ad2.14_ACCACTGT	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
17	Ad2.15_TGGATCTG	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
18	Ad2.16_CCGTTTGT	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
19	Ad2.17_TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
20	Ad2.18_GAGGGGTT	CAAGCAGAAGACGGCATACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
21	Ad2.19_AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT
22	Ad2.20_GTGTGGTG	CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGT
23	Ad2.21_TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
24	Ad2.22_TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
25	Ad2.23_TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT