ATAC-seq library prep

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Purpose and Background

Making ATAC-seq libraries from INTACT-purified nuclei. ATAC = Assay for Transposon Accessible Chromatin. Open chromatin allows insertion of the transposase, and the PCR reaction amplifies from transposon to transposon, across open chromatin areas. These open areas represent euchromatin (=lightly packed) regions, and furthermore the linker regions between nucleosomes and areas where transcription factors are not bdiding.

Materials

- Nextera library Kit (Illumina FC-121-1030)
- ~~Qiagen MinElute PCR purification kit (Qiagen 28004)~~ **use AmpureXP beads**
- NEBNext High-Fidelity 2X PCR master mix (NEB M0541)
- 100× SYBR Green I (Invitrogen, cat. no. S-7563)
- NEBNext Library Quant Kit for Illumina (NEB E7630S) – **NB: can be replaced by plate reading setup**
- RNAse free PCR strips
- Magnetic rack for PCR strips
- PCR machine

Buffers and solutions:

- 70% ethanol
- RNease-free water

Procedure

1. Transposition reaction and purification

1.1) Prepare the transposition reaction mix on ice according to the following table and mix well:

Component	Vol (µl)
2X TD buffer	25
Water	22.5
TDE1 transposase	2.5
Total	50

1.2) To a 0.2 ml PCR strip on ice, add 20,000 to 50,000 bead-bound nuclei from INTACT.

1.3) Place the PCR strip on a magnet to capture nuclei+beads, decant supernatant, and resuspend the nuclei+beads in 50 µl of transposition mix from step B1.

1.4) Place the transposition reaction at 37° C for 30 min with occasional gentle mixing.

1.5) Purify transposed DNA using the Qiagen MinElute PCR purification kit (Note: it is likely that AmpureXP clean up can be substituted here.) and elute in 11 µl of elution buffer EB. DNA can now be stored at -20° C if necessary.

1.5) ALTERNATIVE Purify transposed DNA using the AmpureXP beads and elute in **11 µl** of 10mM Tris. DNA can now be stored at -20° C if necessary.

Component	volume (ul)
transposed DNA	51

Ampure beads (1.8X)	91.8
Total	

2) PCR amplification of the library

2.1) Combine all reactants in a 0.2 ml tube on ice according to the following table:

Component	Volume (µl)
Transposed DNA (from step B5)	10
Water	10
25 µM ATAC Primer 1	2.5
25 µM ATAC barcoded Primer 2*	2.5
2X NEBNext high fidelity PCR mix	25
Total volume	50

If this is the first time you carry out this protocol, follow all the steps 2.2 – 2.7. If you have optimized the number of cycles for your samples, you can do step 2.2 with the cycle number you know to be good for your material and skip steps 2.4 - 2.7.

2.2) Perform thermal cycling according to the following table:

Cycle number	Temperature (˚C)	Time
1	72	5 min
	98	30 sec
5+X cycles	98	10 s
	63	30 s
	72	1 min
	4	hold

2.3) Place reactions on ice once the thermal cycler reaches 4˚C.

2.4) Set up a qPCR to determine the number of additional cycles needed to amplify the library. Assemble all reactants on ice – divide into two technical reps?

Component	Volume (µl)
Previous PCR (from step 2.3)	5
Water	3.6
25 µM ATAC Primer 1	0.25
25 µM ATAC barcoded Primer 2	0.25
10X SYBR green (diluted from 100X)	0.9
2X NEBNext high fidelity PCR mix	5
Total volume	15

2.5) Perform thermal cycling in the qPCR machine according to the following table:

Cycle number	Temperature (˚C)	Time
1	98	30 sec
20 cycles	98	10 s
	63	30 s
	72	1 min

2.6) To determine the optimal number of cycles to run the remaining 45 µl of each PCR from step 2.3, view the linear Rn versus cycle number plot on the qPCR machine. Determine the cycle number at which the fluorescence for a given reaction is at 1/3 of its maximum, and use this number of additional cycles (*N*) for each reaction from step 2.3.

2.7) Run the remaining 45 µl of each PCR from step 2.3

Cycle number	Temperature (˚C)	Time
1	98	30 sec
N cycles	98	10 s
	63	30 s
	72	1 min
	4	hold

3) Clean up and pooling of libraries

3.1) Run 2ul of the libraries on a gel to visualize the library sizes. If the libraries are all under 670bp (unlikely), replace the next step with regular Ampure XP clean up protocol.

3.2) Ampure XP bead "upper cut" protocol to remove fragments over 670 bases:

> Please note: It is recommended to verify this protocol first with <u>your batch of Ampure beads</u>. This selection protocol will also remove adapter dimers, if they are not dominating the library.

1. Follow the standard Ampure XP handling extractions from the manufacturer (e.g. equilibrate the beads at to room temperature before use; vortex beads before use)
2. Add 0.55 volumes (i.e 26.4ul for 48ul of enrichment product) of resuspended Ampure beads to your sample, mix, incubate for 5 minutes at RT.
3. Collect the beads on a magnet.
4. Transfer the supernatant to a new tube.
5. Add another 1x original volume Ampure beads (i.e. 48ul for 48ul of enrichment product) to the supernatant; mix; incubate for 5 minutes.
6. Collect the beads on a magnet
7. Carry out the regular 70% ethanol washes and the sample elution into 20ul of Tris.
8. DNA can now be stored at -20° C.

3.3) Run 2ul of the libraries on a gel to visualize the library sizes and verify that there are no primers left in the libraries. If there are still primers in the libraries, carry out another Ampure XP cleanup:

- Add 1.5 volumes (i.e. 27 ul for 18 ul of enrichment product) resuspended Ampure beads to sample, mix well and let sit at room temperature for 5 minutes.
- Place on magnetic tray and remove supernatant
- Wash 2X with 200 ul of 80% EtOH without resuspending pellet. Allow pellet to dry.
- Re-suspend pellet in 20uL 10mM Tris pH 8.0 and re-suspend beads by pipetting up and down 10 times. Incubate at RT for 2min.
- Magnetize and transfer the supernatant into fresh tubes.
- 2 ul can be run on a gel to make sure of sufficient recovery and complete lack of adapter contamination.
- Proceed to quantification and pooling

3.4) Quantify the libraries using either
> the NEBNext library quantification kit for Illumina
> OR plate reader (Kaisa prefers this)

3.5) Pool libraries based on concentrations from step 3.4 and verify pool size on Bioanalyzer prior to sequencing. If primers or large library fragments persist, repeat size-selection or AmpureXP cleanup.