Blueprint: Assay for Transposase Accessible Chromatin (ATAC-seq)

To obtain transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position (Buenrostro et al, 2013).

1. Cell Preparation

1. Harvest cells (no fixation), protocol to be defined by the user.
2. Spin down 100.000 cells at 800 x g for 5 min at 4˚C.
3. Wash once with 50 µL of cold 1x PBS buffer. Spin down at 800 x g for 10 min at 4˚C.
4. Gently pipette to resuspend the cell pellet in 50 µl of cold lysis buffer.
5. Spin down immediately at 800 x g for 10 min at 4˚C.
6. Discard the supernatant, and immediately continue to transposition reaction.

2. Transposition Reaction and Purification

1. Make sure the cell pellet is set on ice.
2. To make the transposition reaction mix, combine the following:

Component	Volume
2x TD Buffer	25 µl
Tn5 Transposes	2.5 µl
NF H2O	22.5 µl
Total volume	**50 µl**

3. Gently pipette to resuspend nuclei in the transposition reaction mix.
4. Incubate the transposition reaction at 37˚C for 30 min.
   Gentle mixing may increase fragment yield.
5. Immediately following transposition, purify using AMPure beads.

3. SPRI AMPure XP purification (ratio sample: beads 1:2)

1. Add 100 µL AMPure beads and pipet up and down 10 times.
2. Incubate 15 minutes at room temperature.
3. Place the eppendorf tubes in the magnetic rack and incubate 2 minutes.
4. Remove the supernatant.
5. Add 200 µl 80% freshly made EtOH.
6. Incubate 30 seconds at room temperature and remove the supernatant.
7. Repeat step 5 and 6.
8. After removing supernatant, shortly centrifuge and put tubes back in the magnetic rack and remove the last amount of supernatant again.
9. Dry for 5 minutes at room temperature.

10. Take out the eppendorf tubes of the magnetic rack.
11. Resuspend the beads into 16.5 µl EB buffer.
12. Incubate 2 minutes at room temperature.
13. Place the eppendorf tubes back into the magnetic rack and incubate 2 minutes at room temperature.
14. Take out 15 µl supernatant (this is your Transposed DNA) and put it into a new Eppendorf tube.

4. Amplification:

1. To amplify transposed DNA fragments, prepare the following PCR reaction mix:

Component	Volume
Transposed DNA	15 µl
100x SYBR Green I**	0.3 µl
NEBNext High-Fidelity 2 PCR Master Mix	25 µl
Nextera Primer index N7.. (25 µM)	2.5 µl
Nextera Primer index S5.. (25 µM)	2.5 µl
NF H2O	4.7 µl
Total volume	**50 µl**

** 10,000x SYBR Green I is diluted in Elution buffer of NGF H2O to make a 100x working solution.

2. Amplify using the following PCR protocol:

Step	Temp	Duration	Cycles
Pre-warming	72°C	5 min	1
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	5
Annealing	63°C	30 sec	
Extension	72°C	1 min	
HOLD	12°C	∞	1

3. In order to reduce GC and size bias in PCR, the PCR reaction is monitored using qPCR to stop amplification prior to saturation. To run a qPCR side reaction, combine the following :

Component	Volume
5 cycles PCR amplified DNA	5 µl
100x SYBR Green I**	0.06 µl
NEBNext High-Fidelity 2 PCR Master Mix	5 µl
Nextera Primer index N7.. (25 µM)	0.25 µl
Nextera Primer index S5.. (25 µM)	0.25 µl
NF H2O	4.7 µl
Total volume	**15 µl**

4. Amplify using the following PCR protocol:

Step	Temp	Duration	Cycles
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	19
Annealing	63°C	30 sec	
Extension	72°C	1 min	
HOLD	12°C	∞	1

5. The additional number of cycles needed for the remaining 45 µl PCR reaction is determined as following:

   (1) To calculate the additional number of cycles needed, plot linear Rn versus cycle and determine the cycle number that corresponds to one-third of the maximum fluorescent intensity (see Fig. A).
   (2) Set 5000 RF threshold
   (3) Calculate the # of cycle that is corresponed to ¼ of maximum fluorescent intensity. See an Example below:

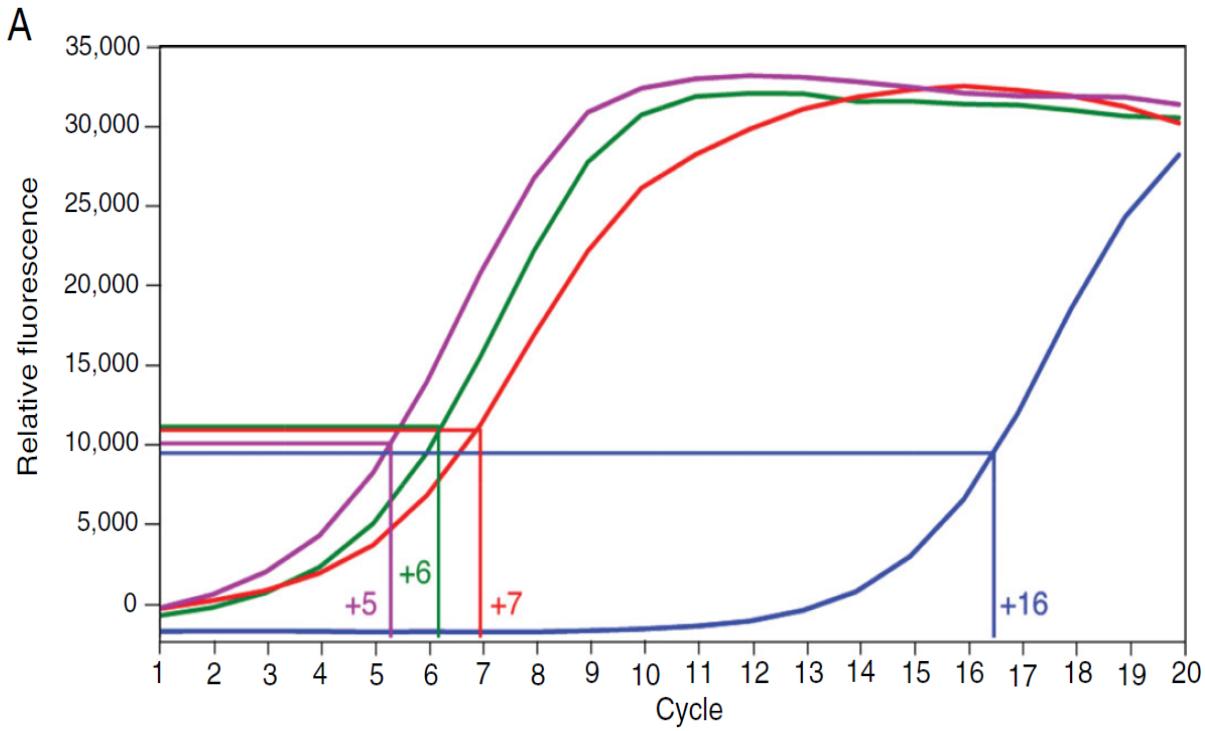

Figure A: Amplification Plot, representative amplification plot demonstrating the correct number of additional cycles to perform for four ATAC-seq libraries.

6. Run the remaining 45 µl PCR reaction to correct # of cycle.

7. Amplify using the following PCR protocol:

Step	Temp	Duration	Cycles
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	#
Annealing	63°C	30 sec	
Extension	72°C	1 min	
HOLD	12°C	∞	1

5. Double SPRI AMPure XP purification:

- *First beads incubation 0,65*

1. Adjust the volume to 50 µl with NF- H2O.
2. Add 32, 5 µl AMPure beads and pipet up and down 10 times.
3. Incubate 10-15 minutes at room temperature.
4. Place the eppendorf tubes in the magnetic rack and incubate 2 minutes.
5. Transfer supernatant to a new Eppendorf tube.
6. *Second beads incubation 1,8x*
7. Add 57, 5 µl AMPure beads and pipet up and down 10 times.
8. Incubate 10-15 minutes at room temperature.
9. Place the eppendorf tubes in the magnetic rack and incubate 2 minutes.
10. Remove the supernatant.
11. Add 200 µl 80% freshly made EtOH.
12. Incubate 30 seconds at room temperature and remove the supernatant.
13. Repeat step 5 and 6.
14. After removing supernatant, shortly centrifuge and put tubes back in the magnetic rack and remove the last amount of supernatant again.
15. Dry for 5 minutes at room temperature.
16. Take out the eppendorf tubes of the magnetic rack.
17. Resuspend the beads into 22.5 µl EB buffer.
18. Incubate 2 minutes at room temperature.
19. Place the eppendorf tubes back into the magnetic rack and incubate 2 minute at RT.
20. Take out 20 µl supernatant (this is your purified sample) and put it into a new Eppendorf tube.

Buffers:

Lysis buffer (100 ml)

Stock	Final concentration	Volume stock solution (ml)
1 M	10 mM Tris-HCL (pH 7,4)	1
5 M	10 mM NaCl	0,2
1 M	3 mM MgCl2	0,3
10 %	0,1 % IGEPAL CA-630	1
MilliQ		Fill up to 100 ml

Store up to 1 week at 4°C

Materials

- 100,000 cells in a single-cell suspension
- Phosphate buffered saline (PBS)
- Lysis buffer (see recipe)
- Molecular biology-grade IGEPAL CA-630 (Sigma-Aldrich, cat. no. I8896)
- TD (2× reaction buffer from Nextera kit; Illumina, cat. no. FC-121-1030)
- TDE1 (Nextera Tn5 Transposase from Nextera kit; Illumina, cat. no. FC-121-1030)
- Nuclease-free H2O (available from various molecular biology suppliers)
- 25 µM Nextera Primer index N7..
- 25 µM Nextera Primer index S5..
- NEBNext High-Fidelity 2× PCR Master Mix (New England Biolabs, cat. no. M0541)
- 100× SYBR Green I (Invitrogen, cat. no. S-7563)
- 100-bp DNA ladder (New England Biolabs; optional)
- Refrigerated centrifuge
- 0.2-ml PCR tubes
- PCR thermal cycler
- qPCR instrument (Applied Biosystems StepOnePlus Real-Time PCR System; cat.
- no. 4376600)
- Typhoon TRIO Variable Mode Imager (Amersham Biosciences; optional)
- Bioanalyzer High-Sensitivity DNA Analysis kit (Agilent; optional)