Fast ATAC-seq

**I Transposition Reaction and Purification**

- count cells

- 25,000-50,000 fresh cells are spun down at 500g 4C for 5min

- remove supernatant

- cells are washed with 1mL Cold PBS (+protease inhibitor)

- spin down at 500g 4C for 5min

- carefully remove all supernatant, keep pellet on ice

- add 25ul-50ul of transposase mixture (on ice), dissociate the pellet by gentle pipetting

|  |  |  |
| --- | --- | --- |
| **Mix:** | **For 50,000 cells** | **For 25,000 cells** |
| 2xTD buffer (Nextera Kit) | 25 ul | 12.5ul |
| TD enzyme (Nextera Kit) | 2,5 ul | 1.25ul |
| Digitonin (20mg/ml # G9441) | 0,5 ul | 0.25ul |
| Nuclease-free water | 22 ul | 11ul |

- incubate @37C for 30min for transposition reaction

- purify DNA immediately with QIAGEN MinElute Reaction Cleanup kit (28204)

- you can keep going or store the DNA in -20

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**II PCR Amplification – Library preparation**

For PCR Amplification, follow the Buenrostro’s protocol with the same reagent.

1. To amplify transposed DNA fragments, combine the following in a PCR tube:

10 μL Transposed DNA

9.7 μL Nuclease Free H2O

2.5 μL 25μM Customized Nextera PCR Primer 1\*

2.5 μL 25μM Customized Nextera PCR Primer 2\* [Barcode]

0.3 μL 100x SYBR Green I\*\* (Invitrogen Cat #S-7563)

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

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

2. 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

3. Monitoring PCR reaction

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:

5 μL 5 cycles PCR amplified DNA

4.44 μL Nuclease Free H2O

0.25 μL 25μM Customized Nextera PCR Primer 1\*

0.25 μL 25μM Customized Nextera PCR Primer 2\*

0.06 μL 100x SYBR Green I

5 μL NEBNext High-Fidelity 2x PCR Master Mix

15 μL Total

\* Complete list of primers available in Section VI of this protocol

4. 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

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

(1) Plot linear Rn vs. Cycle

(2) Set 5000 RF threshold

(3) Calculate the # of cycle that is corresponded to ¼ of maximum fluorescent intensity

Here is an example:

Clean samples with MiniElut PCR purification Kit (Qiagen 28004/28006)

**III size selection**

- make a 2% agarose gel with 1xTAE

- load samples and run the gel at 150V for 1h

- cut the gel between 150-600bp

-extract gel with QIAGEN Kit(28604)

- measure the concentration with Qubit and run samples on the lab-on-chip.