# ATAC-seq method

## 1. Cell activity assay

Cell activity is detected with Trypan blue assay and counted. (For cell samples)

## 2. ATAC-seq library preparation

ATAC-seq library construction is performed as previously reported (Bajic et al., 2018; Buenrostro et al., 2013; Corces et al., 2017). Briefly, nuclei are extracted from samples, and the nuclei pellet is resuspended in the Tn5 transposase reaction mix. The transposition reaction is incubated at 37°C for 30 min. Equimolar Adapter1 and Adapter 2 are added after transposition, PCR is then performed to amplify the library. After the PCR reaction, libraries are purified with the AMPure beads (Figure 1).

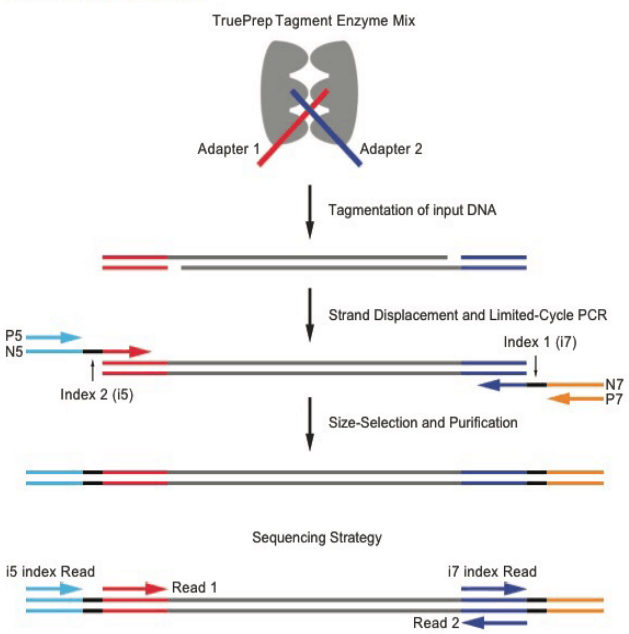


Figure 1 Library construction workflow

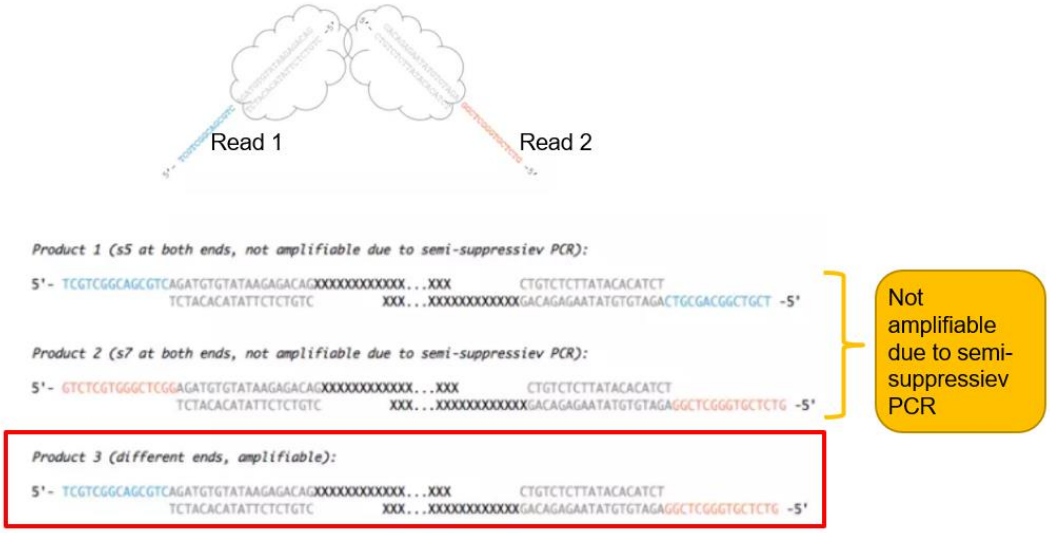


Figure 2 Details of Tn5 generated library

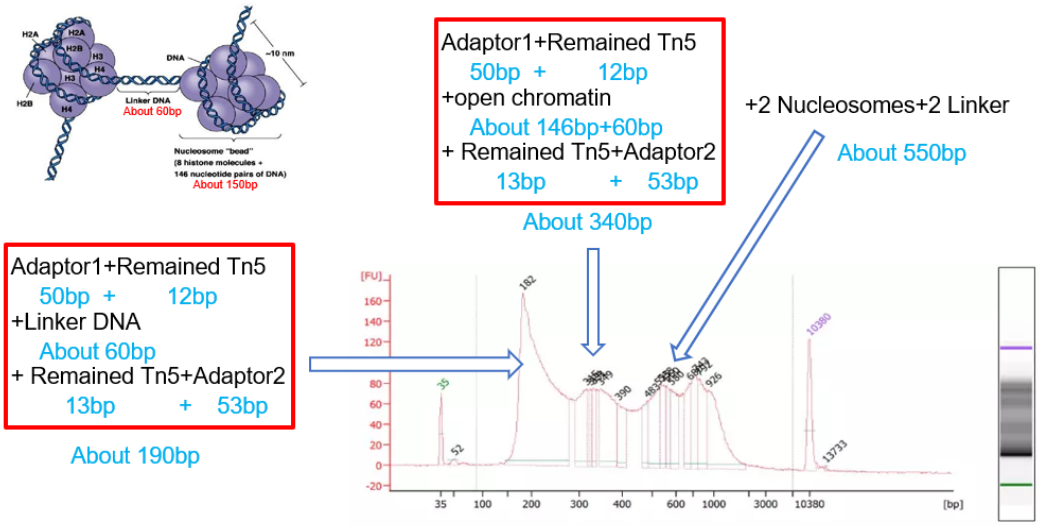


Figure 3 Size selection of ATAC-seq library

## 3. Quality control of Library

After the construction of the library, the initial quantification was done with Qubit 2.0, and the library is diluted to 1ng/l. Then the insertion size of the library is detected with NGS3K. If meeting the expectation, the accurate concentration of the library is quantified by Q-PCR (library effective concentration > 2nM) to ensure the accurate molar amount that will pooled for sequencing.

## 4. Sequencing

After library quality control, sequencing is performed for different libraries according to the concentration and the demand of data amount on Illumina NovaSeq platform. The basic principle of sequencing is sequencing by synthesis. Four kinds

of fluorescent labeled dNTP, DNA polymerase and adapter primers are added to the flow cell for amplification. When each sequence cluster extends the complementary chain, each fluorescent labeled dNTP releases the corresponding fluorescence. The sequencer captures the fluorescent signal and converts the light signal into the sequencing peak through computer software to obtain the sequence information of the fragment to be detected. The sequencing process is shown in the following figure (Figure 2).

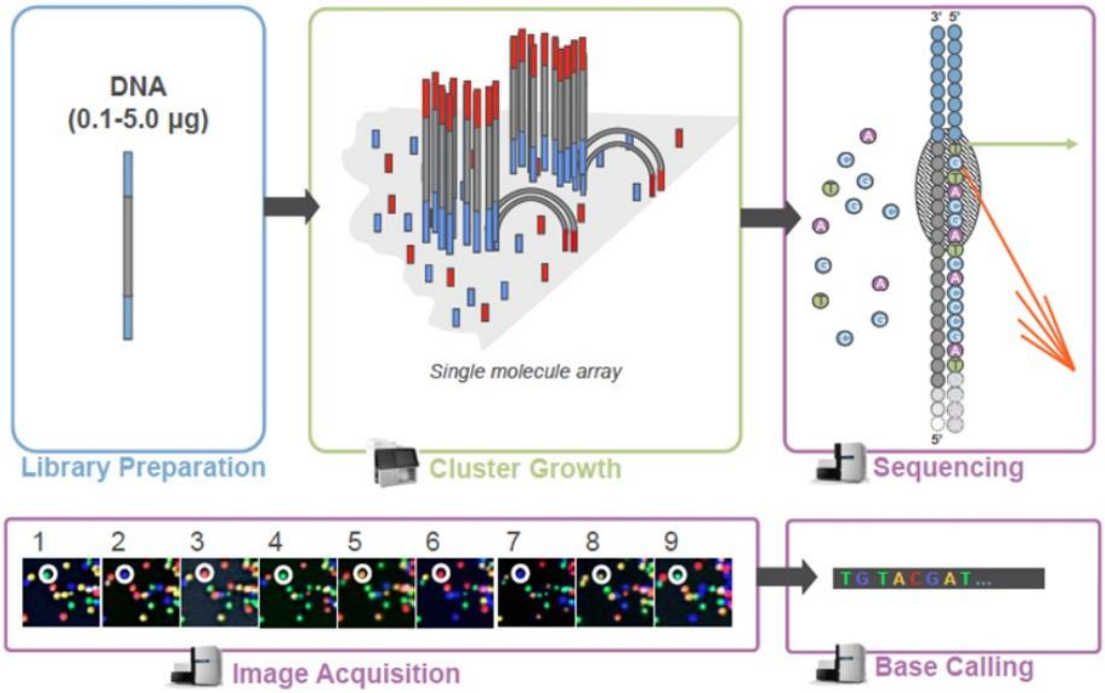


Figure 2 The sequencing principle and process

## Reference


Bajic, M., Maher, K. A., & Deal, R. B. (2018). Identification of open chromatin regions in plant genomes using ATAC-Seq. *Plant Chromatin Dynamics* (page 183–201). Springer.

Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y., & Greenleaf, W. J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nature methods*, *10*(12), 1213–1218.

Corces, M. R., Trevino, A. E., Hamilton, E. G., Greenside, P. G., Sinnott-Armstrong, N. A., Vesuna, S., Satpathy, A. T., Rubin, A. J., Montine, K. S., & Wu, B. (2017). An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. *Nature methods*, *14*(10), 959–962.