ACTIVE MOTIF®

Enabling Epigenetics Research

ATAC-Seq Spike-In Control

Catalog No. 53173

(Version A1)

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Overview

Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-Seq) has emerged as a powerful method for investigating open or accessible chromatin across the genome. However, the identification of differences between data sets can be challenging when global modification changes occur, such as in the case of studying the effects of chromatin modifying enzyme inhibitors. Additionally inaccurate quantification of starting material or technical variation during processing results in variation across sample data. Currently available bioinformatic-based normalization methods are not applicable in these instances, and the only reliable way to overcome bias and variation is to add a known standard (Spike-In) into all samples. Active Motif offers Spike-In reagents for ChIP-Seq and CUT&Tag, and has now introduced a similar approach for ATAC-Seq.

Active Motif's strategy for ATAC-Seq normalization is to Spike-In cryopreserved *Drosophila* cell nuclei into samples prior to nuclei prep and tagmention. During tagmentation both the test cells and the *Drosophila* nuclei are tagged at open chromatin consistently across all samples. A normalization factor is then created based on the *Drosophila* signal and applied to the test genome.

The ATAC-Seq Spike-In Control kit works with the ATAC-Seq Assay Kit (Catalog No. 53150).

Product	Format	Catalog No.
ATAC-Seq Spike-In Control	16 rxns	53154

Kit Components and Storage

All components are guaranteed for 6 months after receipt when stored properly.

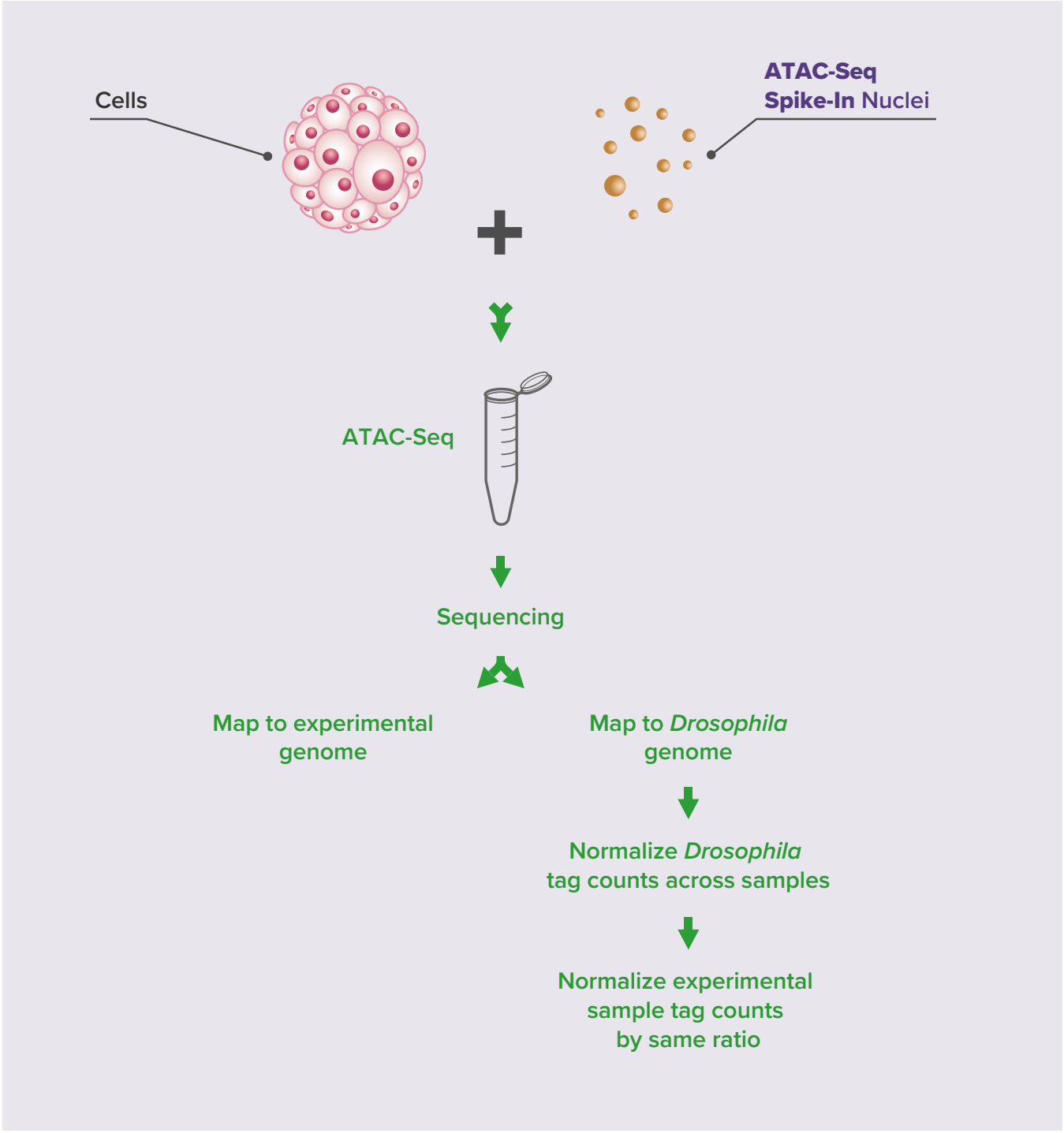
Kit Component	Quantity	Storage
ATAC-Seq Spike-In Nuclei	4 x 80 µL	-80°C

ATAC-Seq Spike-In Nuclei are provided as four 80 µL vials of nuclei at a concentration of 500 nuclei/µL.

We recommend using 10,000 ATAC-Seq Spike-In Nuclei per 50,000 to 100,000 cells used in an ATAC-Seq Assay Kit reaction. For less than 50,000 cells, 10,000 ATAC-Seq Spike-in Nuclei can be used but will amount to more than 5% of sequencing reads.

Note: If more than 1 vial of nuclei are used across an experiment, pool the needed vials of nuclei together to ensure consistency in the amount of nuclei added per reaction for spike-in.

Workflow


Cells
ATAC-Seq
Spike-In Nuclei
ATAC-Seq
Sequencing
Map to experimental genome
Map to *Drosophila* genome
Normalize *Drosophila* tag counts across samples
Normalize experimental sample tag counts by same ratio

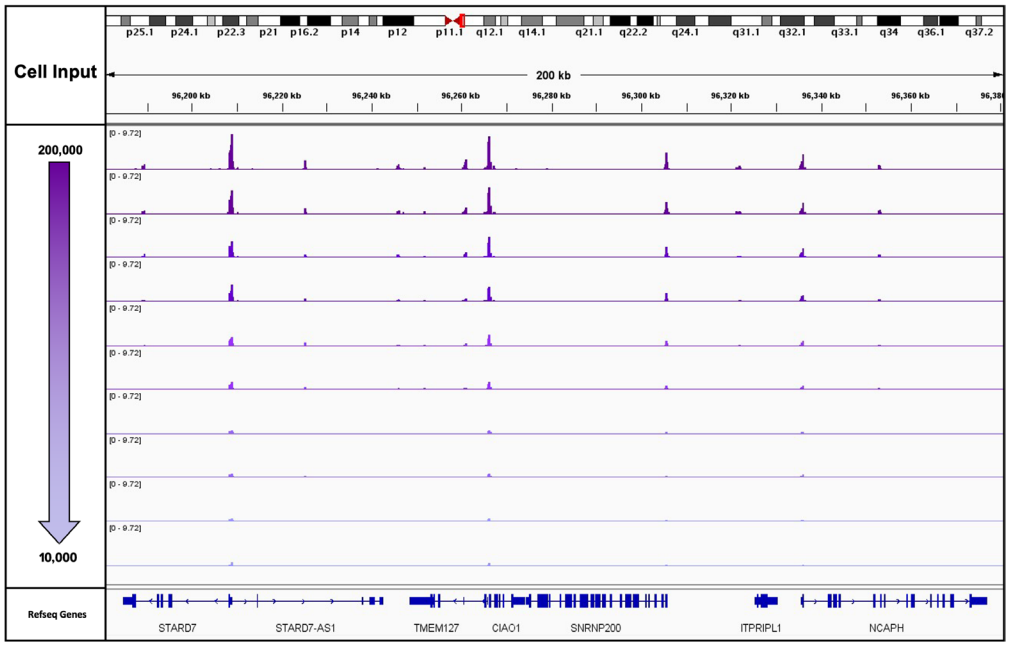


Figure 1: K562 Starting Cell Numbers for ATAC-Seq
This experiment used 10,000 drosophila nuclei as a spike in for a mock treatment. The mock treatment was a cell titration performed in duplicate from 200,000 cryopreserved K562 cells down to 10,000 cryopreserved K562 cells. The actual cell numbers are 200,000, 100,000, 50,000, 20,000 and 10,000 cells. This figure is all of the normalized tracks for the titration in duplicates.

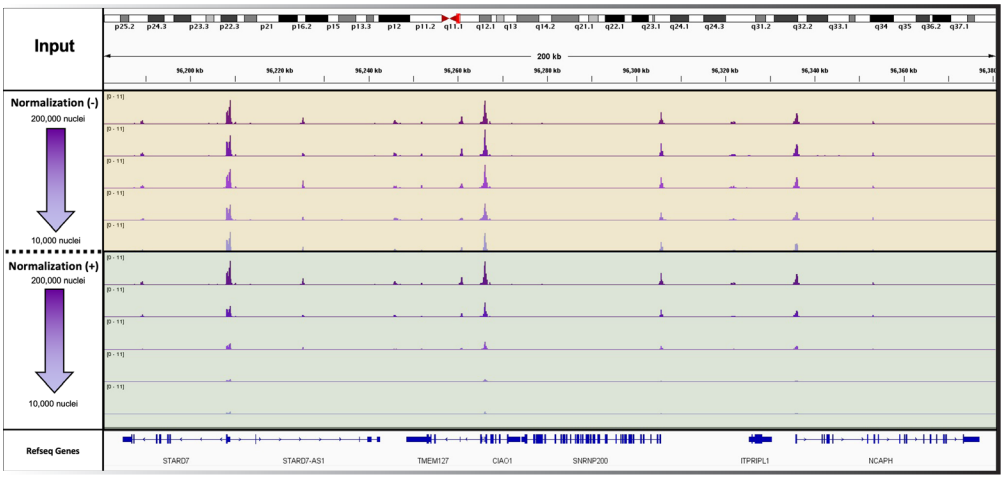


Figure 2: K562 Starting Cell Numbers for ATAC-Seq with and without Spike-In Normalization
This experiment used 10,000 drosophila nuclei as a spike in for a mock treatment. The mock treatment was a cell titration performed in duplicate from 200,000 cryopreserved K562 cells down to 10,000 cryopreserved K562 cells. The actual cell numbers are 200,000, 100,000, 50,000, 20,000 and 10,000 cells. This figure is both the spike-in normalized and unnormalized tracks for one of the duplicates in the titration.

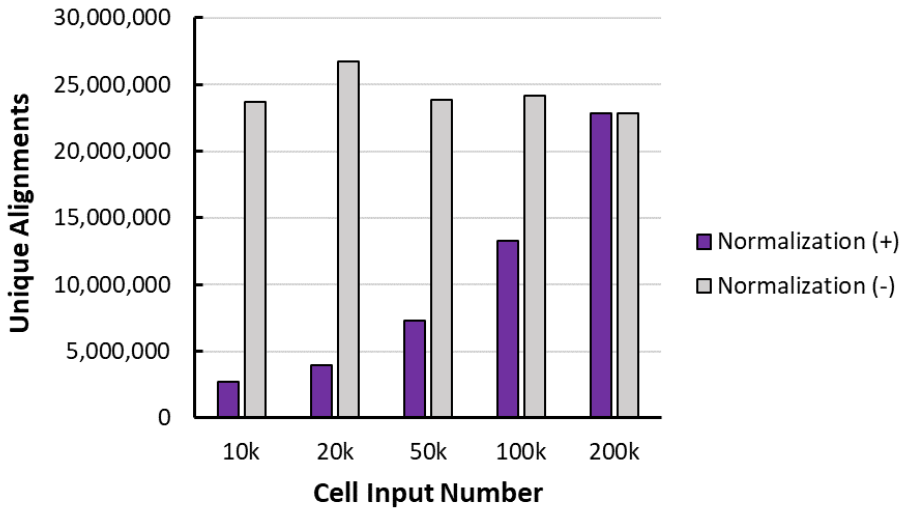


Figure 3: K562 Paired Alignments with and without Normalization
This is the unique alignments (to human genome) for one of the duplicates, before (-) and after (+) normalization with the spike in, for each of the cell inputs.

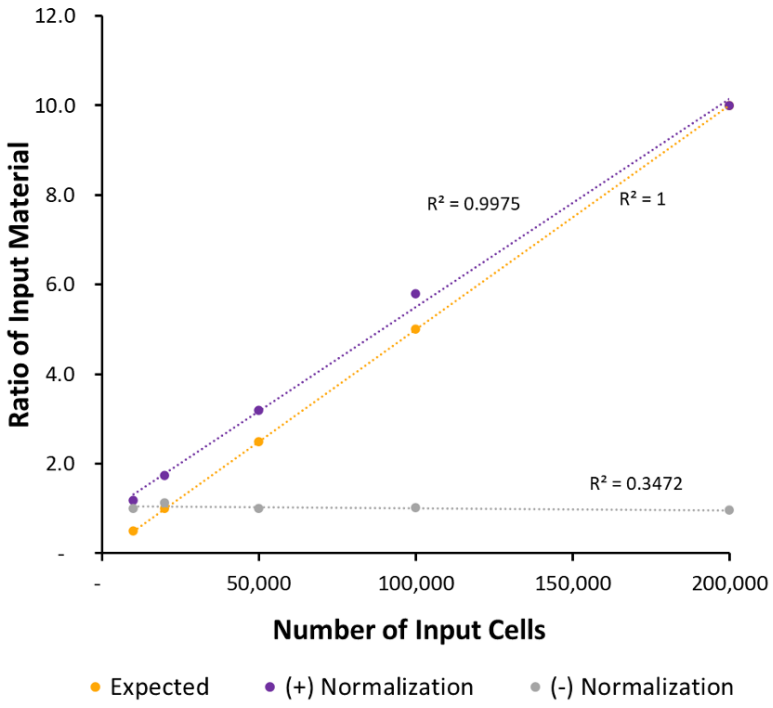


Figure 4: K562 Paired Alignments After Normalization to *Drosophila* Paired Alignments
This is the ratio of input material. For the sample data (normalized and unnormalized) this was calculated by dividing the number of unique fragments aligning to the human genome for each sample by the number of unique alignments for the max input. For the expected sample – the number of cell input was divided by the max number of cell input. (numbers are multiplied by 10 to increase scale).

The amount of cryopreserved Drosophila nuclei used per ATAC-Seq reaction may need to be optimized with the goal of having Drosophila reads make up only 2-10% of the total sequencing reads. However, when using 50,000-100,000 cells we recommend using 10,000 Drosophila nuclei. For samples that have less than 50,000 cells, 10,000 nuclei can be used, but will account for more than 5% of total reads. This can be optimized by titrating the number of Drosophila nuclei.

To demonstrate the utility of this approach, chromatin accessibility differences were mimicked by setting up ATAC-Seq reactions with different amounts of starting cell numbers (Figure 1). Various numbers of cryopreserved human K562 cells (10,000, 20,000, 50,000, 100,000, and 200,000) were combined with 10,000 of cryopreserved Drosophila nuclei for each experiment.

Libraries were quantified and sequenced to a depth of 40-50 million reads per sample. However, sequencing to equal read depth for each sample masks the differences in starting amounts for each sample (Figure 2). Therefore Spike-In normalization is required to reveal the differences in starting material[2]. For normalization, the sample with the lowest number of Drosophila reads (Figure 3) was used to generate normalization factors across samples, which were then applied to down-sample the human read counts for each sample accordingly (Figure 4). After obtaining normalized human read counts, a standard ATAC-Seq pipeline was used for peak calling generation of bigWigs.

ATAC-Seq Normalization Guidelines

1. Perform ATAC-Seq by combining the ATAC-Seq Spike-In Nuclei with the experimental sample cells to be used in ATAC-Seq prior to wash and lysis steps. Perform ATAC-Seq as per the instructions provide in Active Motif's ATAC-Seq Assay kit (Catalog No. 53150). Use the guidelines provided for the number of Spike-In nuclei that should added to the experimental sample based on cell number.
2. Sequence the ATAC-Seq libraries using an NGS platform.
3. Map the ATAC-Seq data to the test reference genome (*e.g.* human, mouse, or other).
4. Map ATAC-Seq data to the Drosophila reference genome.
5. Count uniquely aligning Drosophila sequence tags and identify the sample containing the least number of tags.
6. Divide the aligned Drosophila tag value from the sample with the lowest Drosophila tag count by the Drosophila tag count value from all other samples to and generate a normalization factor for each sample. (Sample 1 with lowest tag count/Sample 2) = Normalization factor. The sample with the lowest drosophila tag count will have a normalization factor of 1.
7. Generate the normalization factors for all samples using the strategy from step 6.
8. Use the normalization factors to down-sample the read counts for each sample.
9. After obtaining normalized human read counts, use a standard ATAC-Seq pipeline starting with the downsampled tag counts for each sample for peak calling and generation of bigWigs.

References

1. Egan, B. *et al.* (2016) *PLoS ONE.* 11(11): e0166438.
2. Taruttis *et al.* (2017) *Biotechniques* 62:53-61

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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