ACTIVE MOTIF®

Enabling Epigenetics Research

CUT&Tag-IT® Spike-In Control, Anti-Mouse

Catalog No. 53173

(Version A1)

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Overview

The Cleavage Under Targets and Tagmentation (CUT&Tag) technique has emerged as a powerful method for investigating the genomic localization of histone modifications of interest. 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 has now introduced a similar approach for CUT&Tag.

Active Motif's strategy for CUT&Tag normalization is to Spike-In cryopreserved *Drosophila* cell nuclei into samples prior to CUT&Tag. Then, during the primary antibody incubation step, a *Drosophila* H2Av antibody is added in addition to the antibody targeting the histone mark of interest. This *Drosophila* H2Av antibody provides a mechanism to reliably tag *Drosophila* histones in a consistent way across all samples. A normalization factor is then created based on the *Drosophila* signal and applied to the test genome. This CUT&Tag Spike-In strategy enables normalization of CUT&Tag data independent of the experimental antibody and without bias.

The CUT&Tag-IT® Spike-In Control, Anti-Mouse works with mouse primary antibodies to the target of interest and the CUT&Tag-IT® Assay Kit, Anti-Mouse (Catalog No. 53165).

Product	Format	Catalog No.
CUT&Tag-IT® Spike-In Control, Anti-Mouse	16 rxns	53173

Kit Components and Storage

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

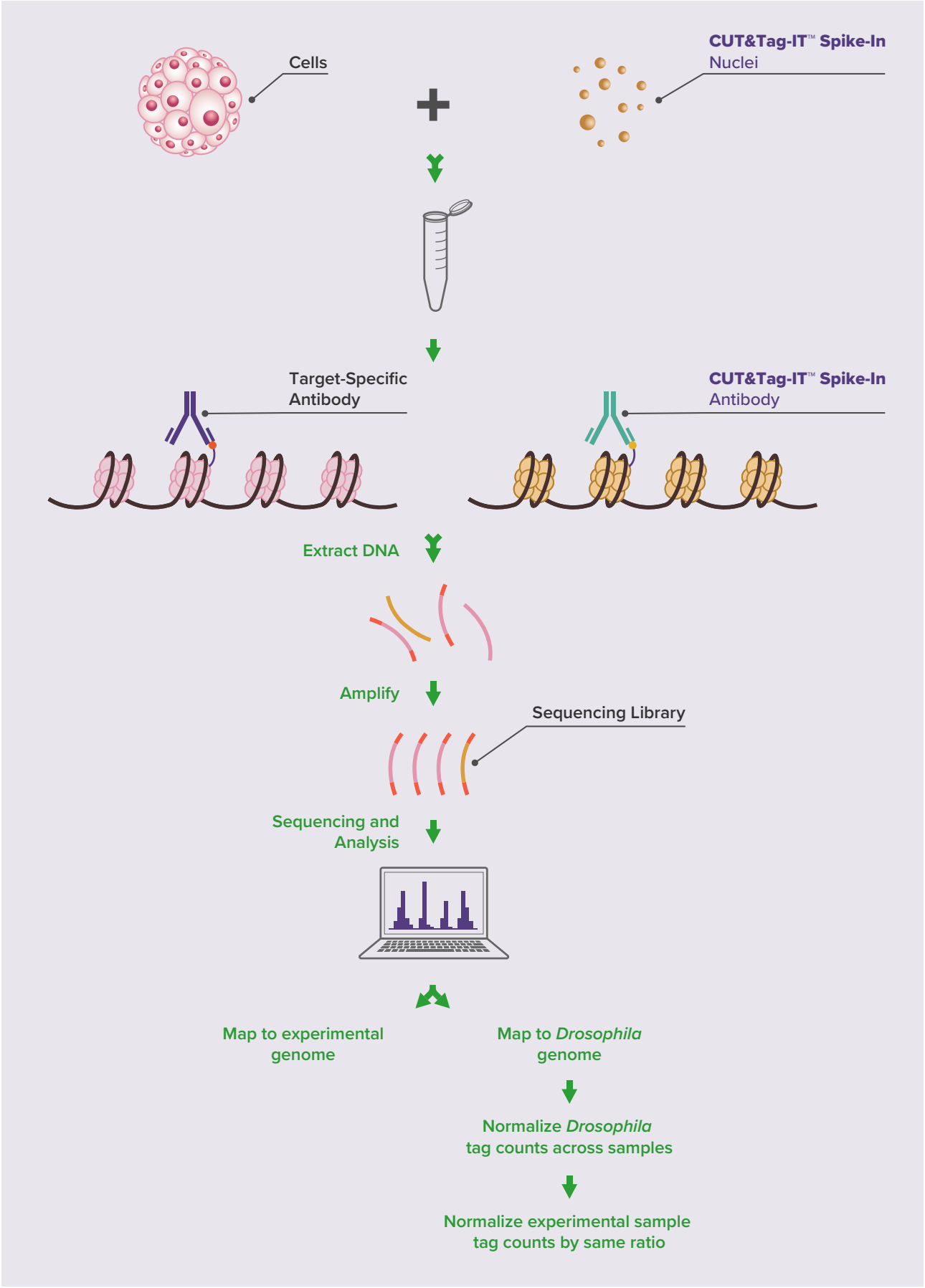
Kit Component	Quantity	Storage
CUT&Tag-IT® Spike-In Antibody, Mouse	16 µL	-20°C
CUT&Tag-IT® Spike-In Nuclei	4 x 160 µL	-80°C

CUT&Tag-IT Spike-In Nuclei are provided as four 160 µL vials of nuclei at a concentration of 500 nuclei/µL.

For abundant histone marks such as H3K27me3 we recommend a Spike-In:sample ratio of 1:10. For less abundant histone marks we recommend a Spike-In:sample ratio of 1:20.

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
CUT&Tag-IT™ Spike-In
Nuclei
Target-Specific
Antibody
CUT&Tag-IT™ Spike-In
Antibody
Extract DNA
Amplify
Sequencing Library
Sequencing and
Analysis
Map to experimental
genome
Map to *Drosophila*
genome
Normalize *Drosophila*
tag counts across samples
Normalize experimental sample
tag counts by same ratio

CUT&Tag Spike-In Reaction Guidelines

Active Motif's CUT&Tag normalization strategy[1] may be applied to any mammalian CUT&Tag assay reaction due to the lack of cross-reactivity of the Spike-In antibody with mammalian samples. The amount of cryopreserved *Drosophila* nuclei and antibody used per CUT&Tag 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 robust antibodies against tightly localized histone modifications, such as H3K4me3, we recommend a Spike-In:test sample ratio of 1:20. For antibodies against spreading marks such as H3K27me3, we recommend at Spike-In:test sample ratio of 1:10.

Conditions	Human Cells	CUT&Tag-IT Spike-In Nuclei	CUT&Tag-IT Spike-In Nuclei/Human Cells Ratio	% DM / Total	
				H3K4me3	H3K27me3
A	50K	20K	1:2.5	29.6%	16.9%
B	100K	20K	1:5	17.4%	8.9%
C	150K	20K	1:7.5	12.6%	6.6%
D	200K	20K	1:10	10.2%	5.0%

To demonstrate the utility of this approach, histone modification level differences were mimicked by setting up CUT&Tag reactions with different amounts of starting cell numbers (Figure 1). Various numbers of cryopreserved human K562 cells (50,000, 100,000, 150,000, and 200,000) were combined with 20,000 of cryopreserved *Drosophila* nuclei for each experiment.

Two histone marks, H3K27me3 and H3K4me3, were evaluated in the CUT&Tag Spike-In assay, with biological duplicates included in 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 CUT&Tag pipeline was used for peak calling generation of bigWigs

Fig. 1

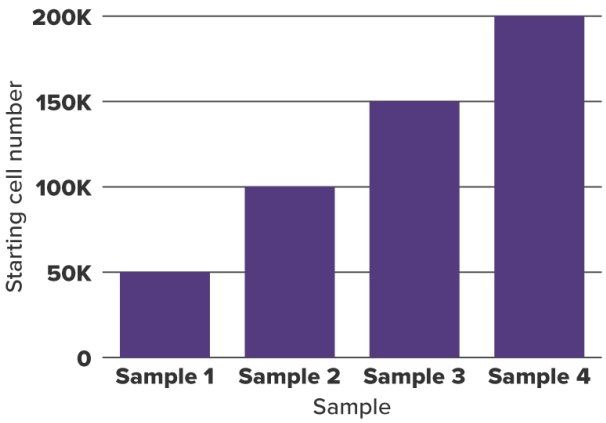


Fig. 2

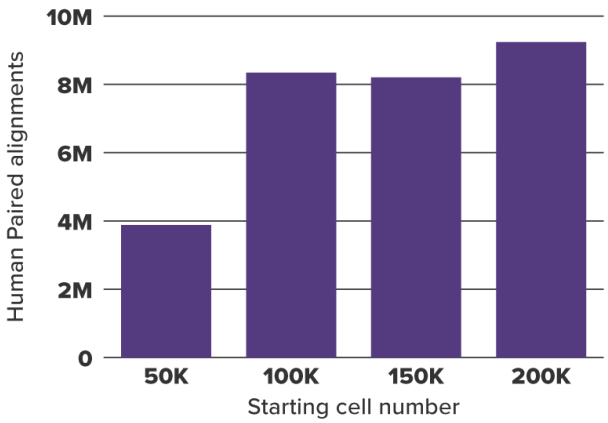


Fig. 3

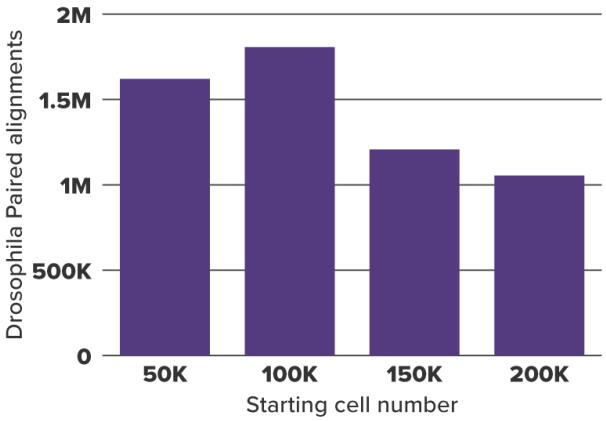


Fig. 4

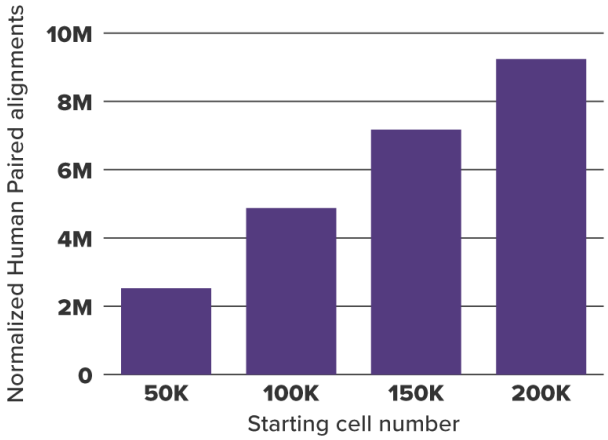


Figure 1: K562 Starting Cell Numbers for CUT&Tag
50,000, 100,000, 150,000, and 200,000 K562 cells were used in CUT&Tag.

Figure 2: K562 Paired Alignments
Paired alignments from duplicate K562 samples were deduplicated with mitochondrial reads removed. 50,000, 100,000, 150,000, and 200,000 K562 samples resulted in 3,879,516, 8,344,823, 8,206,876, and 9,235,143 reads. The number of alignments is not reflective of the number of cells added to the experiment, thus normalization is needed.

Figure 3: *Drosophila* Paired Alignments to Obtain Normalization Factor
20,000 CUT&Tag-IT Spike-In Nuclei were added to 50,000, 100,000, 150,000, and 200,000 K562 samples and assayed in CUT&Tag. The resulting *Drosophila* alignments for 50,000, 100,000, 150,000, and 200,000 K562 samples were 1,620,351, 1,805,662, 1,206,376, and 1,054,504. In general, the more K562 cells in the experiment, the less *Drosophila* alignments resulted because more reads were going to K562 cells. The sample with the lowest number of reads, in this case 200,000 K562 cells, was used for the normalization factor in Figure 4.

Figure 4: K562 Paired Alignments After Normalization to *Drosophila* Paired Alignments
K562 paired alignments were normalized to the *Drosophila* alignment number from 200,000 K562 cells shown in Figure 3. Now the expected ratios based on amount of starting cells has been restored.

CUT&Tag Normalization Guidelines

1. Perform CUT&Tag by combining the CUT&Tag-IT® Spike-In Nuclei with the experimental sample cells to be used in CUT&Tag. Perform CUT&Tag as per the instructions provide in Active Motif's CUT&Tag-IT® Assay Kit, Anti-Mouse (Catalog No. 53165) and add 1 µL of CUT&Tag-IT® Spike-In Antibody, Mouse in combination with your test antibody, during the primary antibody incubation step. Use the guidelines provided for nuclei and antibody quantities based on the antibody target.
2. Sequence the CUT&Tag libraries using an NGS platform.
3. Map the CUT&Tag data to the test reference genome (*e.g.* human, mouse, or other).
4. Map CUT&Tag 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 CUT&Tag 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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