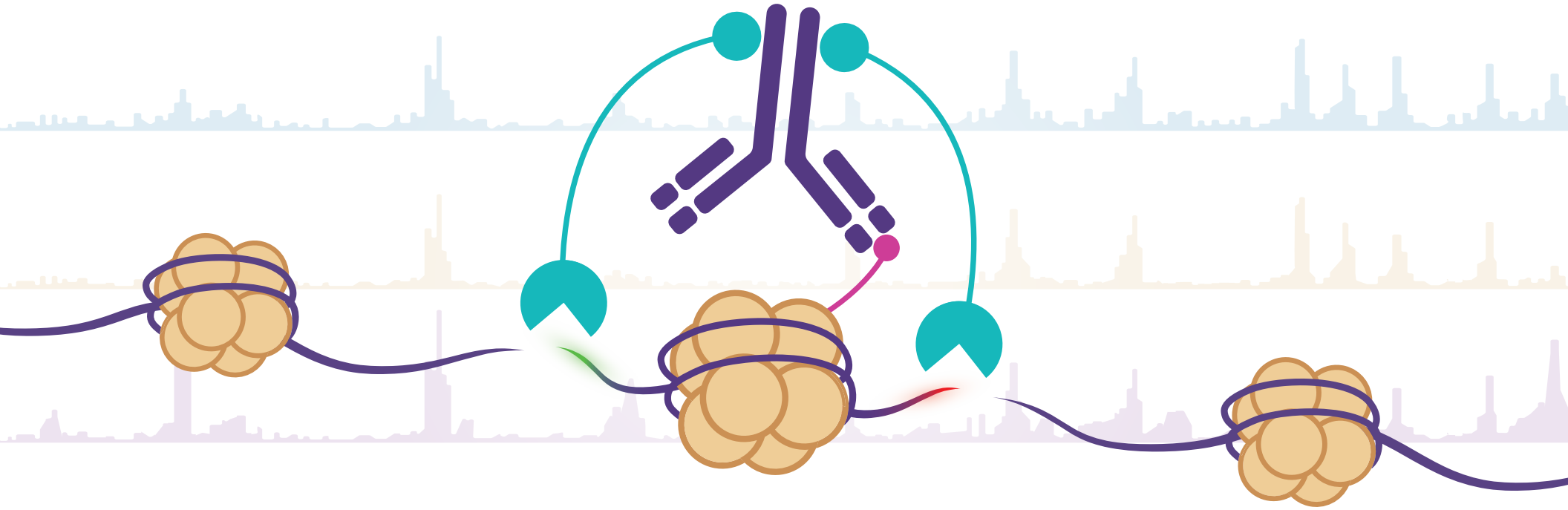
CUT&Tag Complete Guide

Method Overview, Protocol and Resources



ACTIVE MOTIF®

Enabling Epigenetics Research

CUT&Tag Complete Guide

Method Overview, Protocol and Resources

The CUT&Tag (Cleavage Under Targets and Tagmentation) method is a variation of Active Motif's patented TAM-ChIP™ technology. CUT&Tag is based on ChIP (Chromatin Immunoprecipitation) principles, antibody-based binding of the target protein or histone modification of interest, but instead of an immunoprecipitation step, antibody incubation is directly followed by the shearing of the chromatin and library preparation. CUT&Tag assays take advantage of a Tn5 transposase that is fused with protein A to direct the enzyme to the antibody bound to its target on chromatin. The Tn5 transposase is pre-loaded with sequencing adapters (generating the assembled pA-Tn5 adapter transposome) to carry out antibody-targeted tagmentation.

CUT&Tag is an invaluable method to investigate genomic localization of histone modifications and some transcription factors that reveals interactions between proteins and DNA or identifies DNA binding sites for proteins of interest. Unlike MNase-Seq or ATAC-Seq methods that target open chromatin and are therefore dependent on chromatin accessibility, the CUT&Tag antibody-based enzyme tethering strategy can target specific histone modifications or proteins to reveal chromatin-binding information that is specific to those sites or proteins of interest. CUT&Tag can rapidly produce high-quality results from less starting material than ChIP-Seq, and enables robust analysis from lower sequencing depths, saving both time and money.

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Overview of CUT&Tag

CUT&Tag, which is short for Cleavage Under Targets and Tagmentation, is a molecular biology method that is used to investigate interactions between proteins and DNA and to identify DNA binding sites for their protein of interest. Although CUT&Tag is similar in some ways to ChIP assays, the starting material for CUT&Tag is live permeabilized cells or isolated nuclei rather than the cells or tissue that are crosslinked with formaldehyde that are used in ChIP.

In CUT&Tag protocols, cells are first permeabilized and incubated with an antibody immobilized on concanavalin A-coated magnetic beads to facilitate the subsequent washing steps. Next, the cells are incubated with a primary antibody specific for the target protein of interest followed by incubation with a secondary antibody. The cells are then incubated with assembled transposomes, which consist of protein A fused to the Tn5 transposase enzyme that is conjugated to NGS adapters. After the incubation, unbound transposome is washed away using stringent conditions. Tn5 is an Mg2+-dependent enzyme so Mg2+ is added to activate the reaction, which results in the chromatin being cut close to the protein binding site and simultaneous addition of the NGS adapter DNA sequences. This leads to chromatin cleavage and library preparation in one single step.

The CUT&Tag method is very sensitive, it has been reported to work with as few as 60 cells for some histone modifications. With ChIP, sonication randomly shears chromatin and the antibody immunoprecipitates DNA with different lengths, usually several hundred base pairs in length. However, with CUT&Tag, the transposase only cuts chromatin at close proximity to the protein binding site, resulting in shorter lengths of DNA being sequenced. This allows lower sequencing depth (3-5 million reads) to generate robust data, with lower background signal than most ChIP-Seq assays. Finally, because the CUT&Tag protocol uses intact cells as the starting material, rather than sonicated chromatin, it can be adapted to single-cell experiments (scCUT&Tag).

CUT&Tag vs. CUT&RUN vs. ChIP-Seq

Many different approaches have been developed to try to improve on crosslinking ChIP (X-ChIP) protocols to produce higher quality results from lower amounts of starting material. In particular, some methods such as Chromatin Endogenous Cleavage (ChEC) and DamID are based on the tethering of nuclease or DNA modifying enzymes to a DNA-binding protein, where the enzyme digests or modifies DNA in the local vicinity of the binding site.

In 2011, Active Motif developed a new method called TAM-ChIP for analyzing protein-DNA interactions. Just like ChIP assays, TAM-ChIP is performed on crosslinked and sonicated chromatin. However, the TAM-ChIP protocol involves using a secondary antibody that is coupled to the Tn5 transposase and NGS adapter sequences. After chromatin capture by agarose beads, Tn5 is activated by Mg2+ to generate the sequencing library index on either side of the protein-binding site, providing higher resolution identification of protein binding sites.

In 2016, Skene and Henikoff developed the CUT&RUN technique. CUT&RUN is short for Cleavage Under Targets and Release Using Nuclease. Similar to the ChEC protocol, CUT&RUN uses the endonuclease and exonuclease properties of the MNase enzyme. For the CUT&RUN protocol, MNase is fused to protein A (pA-MNase) to guide the chromatin cleavage to antibodies bound to the protein targets of interest at their binding sites across the genome. CUT&RUN starts with isolated nuclei from live cells that are immobilized on lectin-coated magnetic beads. The nuclei are then incubated with an antibody specific for the protein of interest and the pA-MNase reagent. The enzymatic reaction is primed by the addition of Ca2+. The protein-DNA complex can be isolated and purified and then used directly for library preparation.

In 2019, Henikoff's lab evolved their CUT&RUN method into CUT&Tag. The CUT&RUN method is good, it can generate high-quality sequencing data from 100-1000 live cells. However, this approach still requires an additional step for adapter ligation prior to library preparation, making it difficult to adapt to single-cell applications. CUT&Tag uses the Tn5 transposase, just like TAM-ChIP, to simultaneously cleave chromatin and insert the NGS adapters for library preparation. This eliminates a time-consuming step in the workflow and also helps CUT&Tag be compatible with smaller amounts of starting material. In summary, CUT&Tag analyzes native chromatin like CUT&RUN and uses antibody-guided tagmentation like TAM-ChIP.

	CUT&Tag-IT™ Assay Kit	CUT&RUN	ChIP-Seq
Performed Under Native Conditions?	Yes	Yes	No
Chromatin Fragmentation Method	Tn5-based tagmentation	MNase digestion	Sonication
Cell Number Requirements	5,000-500,000 cells	500,000 cells	1-10 million cells
Sequencing Depth Required *	2 million reads **	8 million reads	20-50 million reads
Integrated Library Preparation?	Yes, uses tagmentation	No, separate library prep required	No, separate library prep required
Compatible Targets	Primarily histone modifications, some transcription factors and co-factors	Wide range of histone modifications, transcription factors, and co-factors	Wide range of histone modifications, transcription factors, and co-factors
Workflow Length	1-2 days	1-2 days	2-3 days

* Kaya-Okur et al. Nature Communications (2019) 10:1930

** For less abundant targets of interest, 8 to 10 million reads are recommended

Single-Cell CUT&Tag (scCUT&Tag)

In the original CUT&Tag article, the team of researchers from the Henikoff lab adapted CUT&Tag to single-cell analysis. Contrary to CUT&RUN, CUT&Tag is adaptable to single-cell because the whole reaction, from antibody binding to library preparation, occurs within intact cells or nuclei.

For adaption to single-cell analysis, the basic CUT&Tag protocol was slightly adjusted. Instead of using magnetic beads to immobilize the cells, the cells are centrifuged between washing. After the tagmentation step, cells are distributed as single cells in nanowells to be barcoded prior to sequencing.

Advantages of CUT&Tag

CUT&Tag is Compatible with Low Cell Numbers

In the publication that first described the CUT&Tag method, the authors went down as low as 60 cells to analyze H3K27me3 profiles across the genome. CUT&Tag is compatible with low cell numbers because pA-Tn5 cleaves DNA directly at the binding site of the antibody and does not require chromatin preparation and sonication steps that can lead to sample loss.

The ability to work with small numbers of cells is an advantage for researchers working on specific cell types, such as rare neuronal populations, pancreatic islets, or stem cells, that are difficult to obtain large numbers of.

CUT&Tag Does Not Require Fixation or Sonication

CUT&Tag is performed on native (unfixed) cells or nuclei, avoiding the need for fixation, chromatin preparation, and sonication steps of standard ChIP workflows. Sonication can be quite challenging to set up and requires specialized equipment that can be expensive. Moreover, over-fixation and over-sonication can destroy protein epitopes preventing them from being immunoprecipitated. Some antibodies work better under native conditions.

CUT&Tag is Fast

Relative to ChIP, which is a multi-step process, CUT&Tag is much faster. Cells are immobilized on magnetic beads and the entire protocol happens in a single tube. The tagmentation step involves chromatin shearing and sequencing library adapter insertion at the same time, which is amenable to high-throughput experiments.

Less Sequencing is Required for CUT&Tag

With CUT&Tag, the chromatin retrieved at the final step of the protocol is in the local vicinity of the binding sites of the protein of interest. The shorter DNA sequences isolated by CUT&Tag means that it doesn't have the same deep sequencing requirements as ChIP-Seq. 3-5 million reads are sufficient to generate robust data following CUT&Tag protocol, which is approximately 10-fold less sequencing than ChIP-Seq assays, which usually require 30-50 million reads per sample. To compare the CUT&Tag signal-to-noise ratios with X-ChIP and CUT&RUN, the 3 protocols were run with the same antibody and sequenced to a depth of 8 million reads. The overall results were quite similar between the ChIP-Seq, CUT&RUN, and CUT&Tag methods. However, ChIP-Seq showed higher background and needed deeper sequencing than CUT&Tag.

CUT&Tag is also more easily adaptable to single-cell analyses than ChIP-Seq and CUT&RUN, making it more useful than the other methods for researchers working with heterogeneous populations, including blood, brain, pancreas or liver.

Limitations of CUT&Tag

CUT&Tag's Native Conditions Are Not Always Suitable

Unfixed/native conditions are not suitable for every experiment. In the original CUT&Tag publication, only histone modifications, NPAT, and CTCF were tested. Many transcription factors are not abundantly expressed, weakly or transiently bind to DNA, or indirectly bind to chromatin. For these cases, chromatin crosslinking and sonication are mandatory steps to detect protein-DNA interactions.

Furthermore, most ChIP-validated antibodies are validated to work with crosslinking conditions and may not work well with native conditions because the protein epitope availability in crosslinked conditions can be different than in native conditions. Therefore, switching from X-ChIP to CUT&Tag would need thorough antibody validation to be certain of antibody specificity and sensitivity in unfixed conditions.

CUT&Tag is Still New

Although it almost seems like CUT&Tag has been around forever and it is catching on quickly, it was only first published in April 2019. Therefore, there is not yet a very long list of papers containing CUT&Tag results published in peer-reviewed journals. Although the publication from the Henikoff lab is quite complete, containing a lot of data comparing CUT&Tag to ChIP-Seq and CUT&RUN, the experimental workflow is very different from ChIP-Seq and data from experiments run with this protocol could be difficult to compare to previous ChIP-Seq data, including the results of the ENCODE project.

CUT&Tag Might Introduce Bias

The Tn5 transposase used in CUT&Tag has a high affinity to open-chromatin regions. Therefore, the CUT&Tag approach might be preferentially suited for analyzing histone modifications or transcription factors associated with actively transcribed regions of the genome and not very well suited for the analysis of regions of the genome that are silenced or contain heterochromatin. Digestion time and pA-Tn5 quantity will need to be carefully optimized for each target to avoid any unspecific tagmentation.

CUT&Tag is a promising technology and time will tell if this method becomes as widely adopted as ChIP-Seq. Like every new protocol, all the flaws have not been detected yet so CUT&Tag optimization must be done with care and every step in the workflow should be checked with suitable controls.

CUT&Tag-IT™ Assay Kit

The kit contains sufficient reagents for 16 CUT&Tag Assay reactions. The kit contains everything required including the assembled pA-Tn5 transposomes. The only additional material required would be the standard lab equipment and reagents which are listed under Additional Materials Required in the manual.

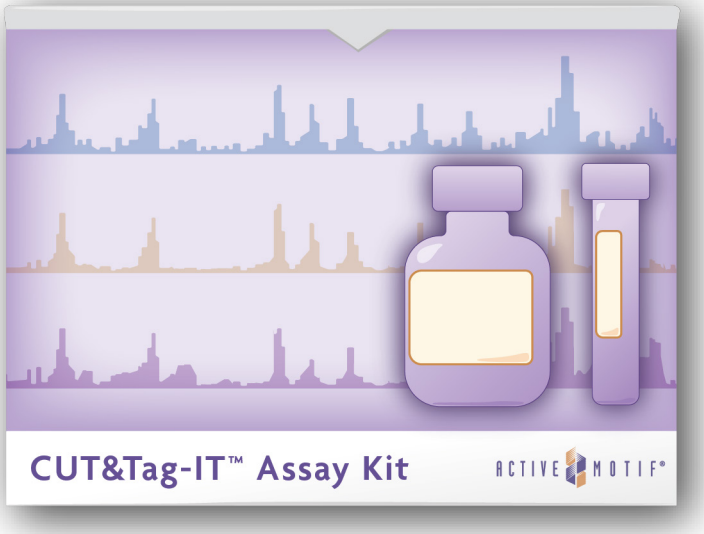
CUT&Tag-IT Assay Kit Advantages

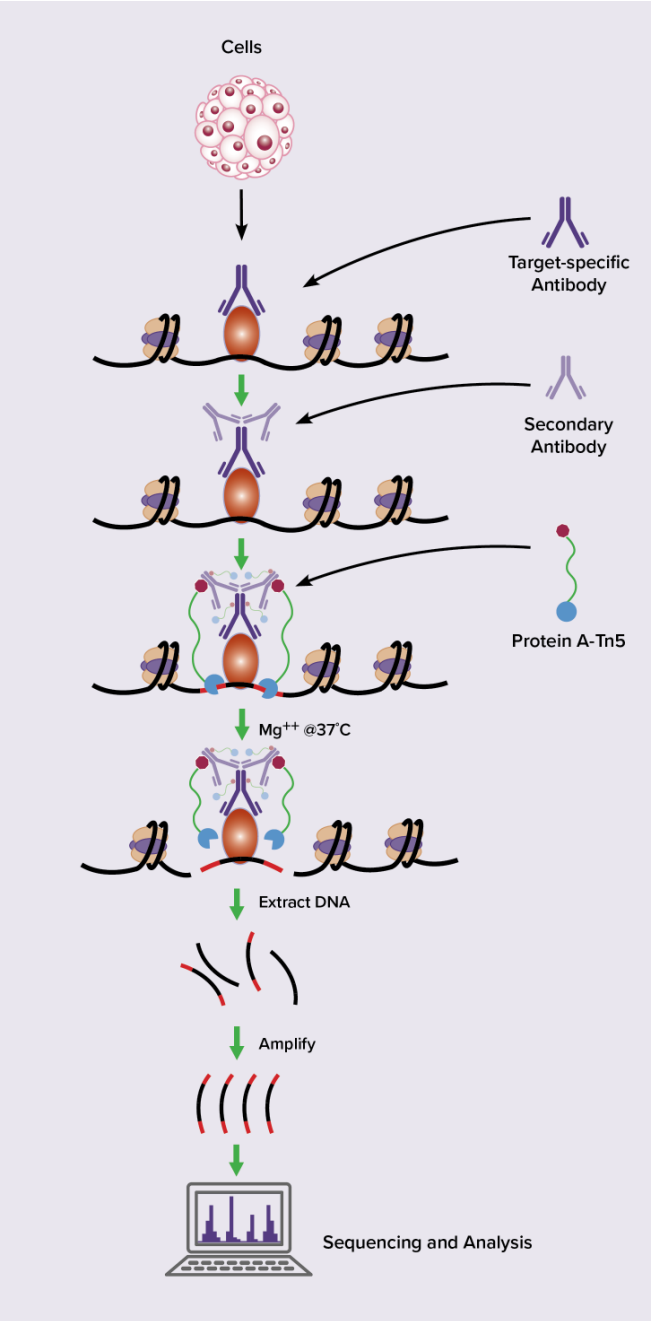
- Compatible with as few as 5,000 cells
- Complete kit with optimized protocol
- Developed for histone marks and some transcription factors
- Sequencing-ready libraries withoug the laborious and costly steps of ChIP-Seq
- Low background signal enables lower sequencing depth
- No artifacts caused by formaldehyde crosslinking

Name	Format	Cat No.
CUT&Tag-IT™	16 rxns	53160

What's Included

- 5% Digitonin, store at -20°C
- Concanavalin A Beads, store at 4°C
- CUT&Tag-IT™ Assembled pA-Tn5 Transposomes, store at -20°C
- Tagmentation Buffer, store at -20°C
- 1X Binding Buffer, store at 4°C
- 1X Wash buffer, store at 4°C
- Dig-Wash Buffer, store at 4°C
- Antibody Buffer, store at 4°C
- Dig-300 Buffer, store at 4°C
- Guinea Pig Anti-Rabbit Antibody, store at -20°C
- Protease Inhibitor Cocktail, store at -20°C
- 0.5M EDTA, store at RT
- 10% SDS, store at RT
- 10 µg/µL Proteinase K, store at -20°C
- DNA Purification Columns, store at RT
- DNA Purification Binding Buffer, store at RT
- DNA Purification Wash Buffer, store at RT
- DNA Purification Elution Buffer, store at RT
- 3M Sodium Acetate, store at RT
- 10 mM DNTPs, store at -20°C
- 5X Q5 Buffer, store at -20°C
- Q5 High Fidelity DNA Polymerase (2U/µL), store at -20°C
- i5 Indexed Primer 1, store at -20°C
- i5 Indexed Primer 2, store at -20°C
- i5 Indexed Primer 3, store at -20°C
- i5 Indexed Primer 4, store at -20°C
- i7 Indexed Primer 1, store at -20°C
- i7 Indexed Primer 2, store at -20°C
- i7 Indexed Primer 3, store at -20°C
- i7 Indexed Primer 4, store at -20°C
- SPRI Beads, store at 4°C





Assay Workflow

- Harvest cells – 30 minutes
- Bind nuclei – 30 minutes
- Bind primary antibody – 2 hours or overnight
- Bind secondary antibody – 1 hour
- Bind CUT&Tag-IT Assembled
- Transposomes – 1.5 hours
- Tagmentation – 1 hour
- DNA extraction – 1 hour
- PCR Amplification – 1 hour
- Next-gen sequencing

DIFFERENCES	ADVANTAGES
NO SONICATION REQUIRED	BETTER RESOLUTION WITHOUT ARTIFACTS FROM FIXATION
USE NATIVE, UNFIXED CELLS	COST SAVINGS – LESS INPUT & LESS SEQUENCING
	TIME TO RESULT – GET DATA IN 1–2 DAYS

Sample Data

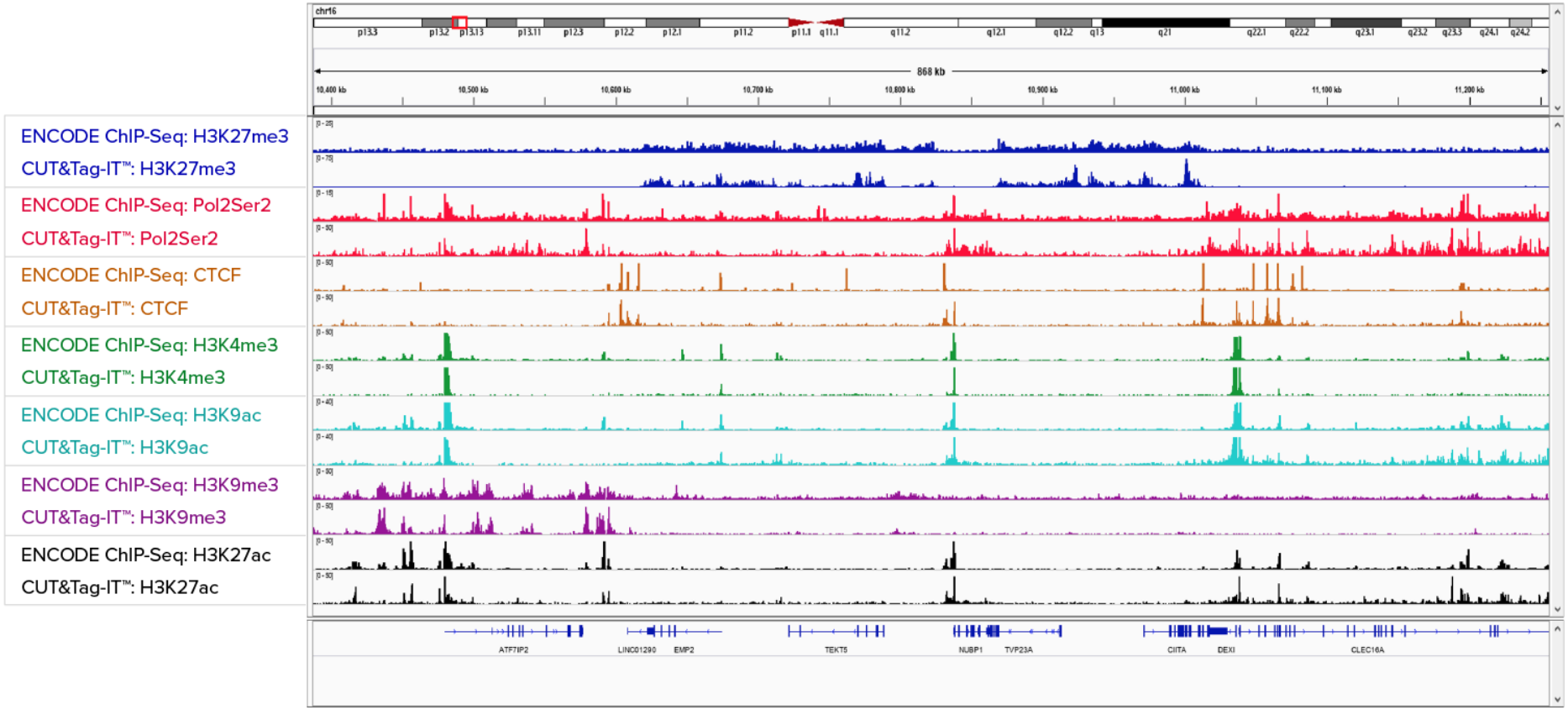


Figure 1: CUT&Tag-IT Assay Kit data correlate well with ENCODE ChIP-Seq data.

Chromatin landscapes from human K562 cells are shown across a 868 kb segment of the human genome region (Chr16: 10,400-11,250) for H3K27me3 (dark blue), phospho-Pol2Ser2 (red), CTCF (orange), H3K4me3 (green), H3K9ac (light blue) H3K9me3 (purple), and H3K27ac (black) showing high concordance of CUT&Tag-IT Assay Kit signals from 100,000 cells compared with ENCODE ChIP-Seq data from at least one million cells. Antibodies used in the CUT&Tag assays for the following targets were: H3K27me3: cat. no 39155, CTCF: cat. no. 61311, H3K4me3: cat. no. 39159, H3K9ac: cat. no. 39917, H3K9me3: cat. no. 39161, H3K27ac: cat. no. 39133.

Supplementary Materials

CUT&Tag Validated Antibodies

Active Motif specializes in manufacturing high-quality antibodies to histones, histone modifications, chromatin proteins and other factors, including a growing list of antibodies that we have experimentally validated in-house to work well in CUT&Tag assays. View a complete list of CUT&Tag-validated antibodies at activemotif.com.

- Histone PTMs
- CTCF, RNA pol II
- AbFlex Recombinant Antibodies

End-to-End CUT&Tag Services

With our CUT&Tag service, you don't need to worry about protocol optimization or testing multiple antibodies to try to find one that works because we take care of all of that for you. There's also no need to learn bioinformatics because analysis of the next-generation sequencing data and explanation of the results are included standard.

Let our team of experts produce high-quality CUT&Tag results from less starting material, saving you time and resources to allow you to focus on the bigger picture. Using our CUT&Tag service is simple. You just submit your samples to Active Motif and receive the analyzed data and publication-ready figures back within a matter of weeks.

Active Motif's End-to-End CUT&Tag service includes:

1. Cell preparation
2. Concanavalin-A incubation & antibody binding
3. Tagmentation using protein-A-Tn5 for library generation
4. Perform next-generation sequencing
5. Comprehensive bioinformatics & data analysis
6. Delivery of publication-ready figures

To learn more, please submit an Epigenetic Services Information Request.

Publications

For an up-to-date list of Active Motif Antibodies Validated or Published for CUT&Tag, visit ActiveMotif.com/publications.

FAQs

Q. Does the CUT&Tag-IT Assay Kit include everything required to perform CUT&Tag?

A. Yes, the kit contains everything required including the assembled pA-Tn5 transposomes. The only additional material required would be the standard lab equipment and reagents which are listed under Additional Materials Required in the manual.

Q. How do I prepare my cells for the CUT&Tag-IT Assay Kit?

A. Follow the protocol in the CUT&Tag-IT™ Assay Kit manual. For adherent cells it is critical that trypsin NOT be used to detach cells as it will damage the epitopes for binding to concanavalin A beads. Use an enzyme-free dissociation method instead, such as scraping or rubber policeman.

Q. Can I use the CUT&Tag-Kit Kit with frozen cells?

A. Cells must be cryopreserved. Flash frozen cell pellets are not compatible with the protocol. To cryopreserve cells we recommend following our Services sample prep protocol.

Q. Do I need to include an IgG control?

A. The purpose of including an IgG control in CUT&Tag experiments is to determine if the pA-Tn5 is specific to genomic regions where the antibody is located/enriched. This negative control is not used in analysis and is different from the INPUT control used in ChIP-Seq. Active Motif R&D found that adding an IgG control does not add any additional value as the pA-Tn5 was shown to be specific. However, if you wish to add an IgG control this is fine.

Q. Are there any QC steps recommended for the CUT&Tag-IT Assay Kit?

A. After library generation, successful library prep quality should be assessed by evaluating the library on a TapeStation or Bioanalyzer. An ideal library would have most of the fragments below 500 bp. We recommend determining the library concentration by using a KAPA Library Quantification Kit.

Q. Does Cut&Tag require an INPUT control like ChIP-Seq?

A. No. CUT&Tag does not require the use of an input control.

Q. Are ChIP-Seq validated antibodies going to work with the CUT&Tag-IT Assay Kit?

A. CUT&Tag and ChIP-Seq have quite different workflows. If an antibody works for ChIP-Seq, that does not necessarily mean it will work in CUT&Tag. We advise using one our CUT&Tag-IT™ Assay Kit validated Active Motif antibodies, or validating a ChIP grade or ChIP-validated antibody.

Q. What controls are recommended to use with the CUT&Tag-IT Assay Kit?

A. A good technical positive control for the reagents and workflow is the antibody H3K27me3 (cat# 39157). For a negative control we recommend using the secondary antibody without the primary antibody. This will show background from the secondary antibody and pA-Tn5 with your samples.

Q. Is the CUT&Tag-IT Assay Kit compatible with both monoclonal and polyclonal antibodies?

A. Yes, it is compatible with rabbit derived antibodies.

Q. Are tagged proteins compatible with CUT&Tag?

A. We have not validated the CUT&Tag-IT™ Assay Kit for tagged proteins. However, in principle, there is no reason why it couldn't work.

Q. Can the standard Active Motif Spike-In Normalization be used for the CUT&Tag-IT Assay Kit?

A. No. Our standard ChIP-Seq Spike-In Normalization method is not compatible with the CUT&Tag-IT™ Assay Kit.

Q. Can I multiplex more than 16 samples using the CUT&Tag-IT Assay Kit?

A. The CUT&Tag-IT™ Assay Kit is supplied with 4x4 unique dual indexes for 16 unique samples. We do not currently have other index primers available. However, the indexed primers in the kit are identical to the Illumina Nextera primers corresponding to N701-N704 and N501-N504. If you would like to multiplex more than 16 samples you could purchase and combine Illumina Nextera primers at the same concentration (25 µM) as those in the kit.

Q. Is the CUT&Tag-IT Assay Kit compatible with qPCR analysis before sequencing?

A. Yes and no. Yes, you can do qPCR, but because of the randomness of Tn5's insertion of the adapter it makes designing primers that will prime a specific gene problematic since it is likely that you will be missing one or the other primer landing site. Therefore, qPCR may contain errors as fragments that do not have the landing site will not be measured.

Q. Are the CUT&Tag-IT Assay Kit libraries single or dual indexed?

A. CUT&Tag libraries are dual-indexed libraries.

Q. Do the CUT&Tag-IT Assay Kit libraries contain Molecular Identifiers?

A. No. CUT&Tag libraries do not contain molecular identifiers.

Q. Should the CUT&Tag-IT Assay Kit libraries be sequenced as single-end or paired-end?

A. The CUT&TAG-IT™ Assay Kit libraries should be sequenced as paired end.

Q. What read length is recommended for the CUT&Tag-IT Assay Kit?

A. We recommend a read length of 2x38 (PE38). This is shorter than the read length described in the Henikoff paper. However, we have not seen an impact on data quality or mapping rates. You can use a higher read length if you wish to.

Q. How do I analyze the sequencing data after using the CUT&Tag-IT Assay Kit?

A. CUT&Tag data is analyzed similarly to ChIP-Seq data. We use a BWA algorithm with peak calling performed using MACS2.

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