Selecting Your Epigenomic Assay: A Guide to ChIP, CUT&RUN and CUT&Tag

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ABSTRACT

For many years, chromatin immunoprecipitation (ChIP) has been a go-to assay for studying histone marks and epigenetic mechanisms. However, two new versatile techniques have recently been developed that are faster, easier, and cheaper than the ChIP assay. Cleavage Under Targets & Tagmentation (CUT&Tag) and Cleavage Under Targets & Release Using Nuclease (CUT&RUN) can be used for probing histone marks and protein-DNA interactions within the natural chromatin context of the cell. Both CUT&Tag and CUT&RUN can be combined with NG-seq to analyze histone modifications and binding of transcription factors, DNA replication factors, or DNA repair proteins at specific target genes or across the entire genome.

CUT&Tag and CUT&RUN are rapid, robust, and true low cell number assays for the detection of protein-DNA interactions. Unlike ChIP, both assays are free from formaldehyde cross-linking, chromatin fragmentation, and immunoprecipitation. When compared to ChIP, CUT&RUN and CUT&Tag require fewer starting cells (as low as 5,000 – 20,000), have a much faster protocol (one day from cells to DNA), and generate lower background signal (require less sequencing depth). In addition, CUT&Tag generates PCR-ready DNA fragments, eliminating the need for adaptor ligation steps during library DNA preparation. We have developed CUT&Tag and CUT&RUN assay kits and internally validated a broad set of antibodies against histone modifications, transcription factors and cofactors that are compatible with each assay.

I will discuss the basics of the CUT&Tag and CUT&RUN assays and important factors to consider when selecting which assay to use for your experiment. In addition, I will provide comparison data showing how these new assays perform versus ChIP and ChIP-seq. Finally, I will discuss the versatility of these assay for mapping various histone modifications, transcription factor, and transcription cofactor binding across multiple sample types, including cells and tissues.

DESCRIPTION

Our newly developed CUT&Tag Assay Kit and CUT&RUN Assay Kit are designed to conveniently provide the reagents needed to perform up to 24 antibody reactions. In addition, standalone products of the critical enzymes used in each assay, loaded pAG-Tn5 and pAG-Mnase, are also available. Both assay kits have been optimized to work in fresh or lightly fixed cells for all types of DNA binding proteins, including histones, transcription factors and cofactors. For analysis of transcription factors and cofactors in tissues, we recommend using the CUT&RUN Assay Kit. If possible, we recommend using 100,000 cells or 1 mg of tissue per reaction for either assay, however we have validated both assays with as few as 5,000 cells for certain target types. Currently, we have internally validated 35+ antibodies for CUT&Tag and 138+ antibodies for CUT&RUN.

Each kit contains positive and negative antibody controls to be included in each experiment. The CUT&Tag Assay Kit is compatible with NG-seq. The CUT&RUN Assay Kit is compatible with both qPCR and NG-seq.

FEATURED CST REAGENTS

CUT&Tag Assay Kit #77552
CUT&Tag pAG-Tn5 (Loaded) #79561
CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415
CUT&Tag PCR Master Mix #63228
CUT&RUN Assay Kit #86652
CUT&RUN pAG-MNase and Spike-In DNA #40366
DNA Purification Buffers and Spin Columns (ChIP and CUT&RUN) #14209
DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795
Multiplex Oligos for Illumina® (Dual Index Primers) (ChIP-seq, CUT&RUN) #47538
Multiplex Oligos for Illumina® (Single Index Primers) (ChIP-seq, CUT&RUN) #29580
35+ CUT&Tag Validated Antibodies
138+ CUT&RUN Validated Antibodies

REFERENCES

Skene, J.S. and Henikoff, S. (2017) eLIFE 6, e21856.
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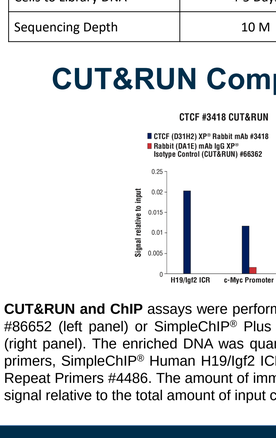
Meers, M.P. et al. (2019) eLIFE 8, e46314.
Meers, M.P. et al. (2019) Molecular Cell 75, 1-14.
Kaya-Okur, H.S. et al. (2019) Nat Commun 10, 1930.
Kaya-Okur, H.S. et al. (2020) Nat Protoc 15, 3264-3283.
Henikoff, S. et at. (2021) Bio Protoc 11, e4043.

CUT&RUN Method Overview



Antibody and pAG-MNase Binding:

1. **Cells are immobilized on Concanavalin A Magnetic Beads to allow for subsequent buffer and reagent exchanges.**
2. **Cell membranes are then permeabilized with digitonin to facilitate the entry of primary antibody and pAG-MNase fusion enzyme into the cell nuclei.**
3. **The target-specific primary antibody recruits the pAG-MNase to the chromatin through protein-protein interactions between the antibody and the pAG domain of the fusion enzyme.**

MNase Digestion:

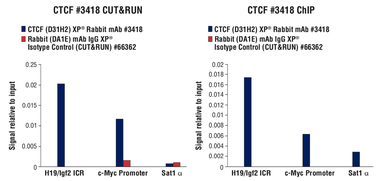
4. **The addition of Ca2+ activates the pAG-MNase, which gently cleaves and liberates the desired chromatin fragments, allowing them to diffuse away from the genomic chromatin, out of the cell, and into the supernatant.**

DNA Purification:

5. **DNA is purified using DNA purification spin columns or phenol/chloroform extraction followed by ethanol precipitation. The purified, enriched DNA is then identified and quantified using qPCR or NG-seq.**

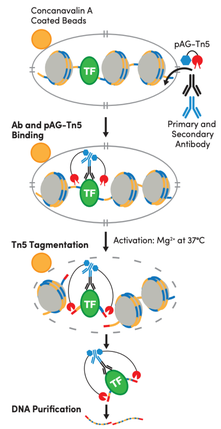
Advantages	ChIP	CUT&RUN	CUT&Tag
Compatible with Histones	✓	✓	✓
Compatible with Transcription Factors	✓	✓	Depends
Compatible with Cofactors	✓	✓	Depends
Starting Material (cells)	4,000,000	5, 000 histones, 10,000 non-histones	5, 000 histones, 20,000 non-histones
Sample Preparation	Cross-link and lyse cells	Bind to conA beads and permeabilize	Bind to conA beads and permeabilize
Chromatin Fragmentation	Non-targeted digestion	Targeted enzymatic digestion	Targeted ligation and tagmentation
Chromatin Enrichment	In vitro	In vivo	In vivo
Compatible with qPCR	✓	✓	X
Compatible with NG-seq	✓	✓	✓
Sample Normalization	X	✓	X
DNA Library Prep	In Vitro	In vitro	In vivo
Cells to Library DNA	4-5 Days	2-3 Days	1-2 Days
Sequencing Depth	10 M	3-5 M	2M

CUT&RUN Comparison to ChIP-qPCR



CUT&RUN and ChIP assays were performed with HCT 116 cells using the CUT&RUN Assay Kit #86652 (left panel) or SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005 (right panel). The enriched DNA was quantified by real-time PCR using human c-Myc promoter primers, SimpleChIP® Human H19/Igf2 ICR Primers #5172, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

CUT&Tag Method Overview



Primary and Secondary Antibody Binding:

1. **Cells are immobilized on Concanavalin A Magnetic Beads to allow for subsequent buffer and reagent exchanges.**
2. **Cell membranes are then permeabilized with digitonin to facilitate the entry of primary and secondary antibodies into the cell nuclei.**

pAG-Tn5 Binding:

3. **The pAG-Tn5 fusion transposase is recruited to the chromatin through protein-protein interactions between the antibodies and the pAG domain of the fusion enzyme.**

Tn5 Tagmentation:

4. **The addition of Mg2+ activates the pAG-Tn5, which cuts targeted genomic DNA and ligates adaptor DNA on site. A further incubation with SDS at 58C breaks open the cellular and nuclear membranes, releasing genomic and adapter bound desired chromatin fragments into the supernatant.**

DNA Purification:

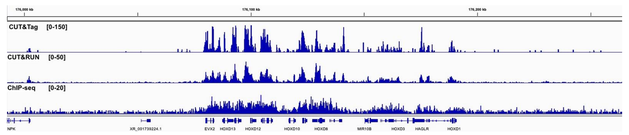
5. **DNA is purified using DNA purification spin columns. The small, adapter bound, desired DNA is then selectively amplified by PCR and analyzed by NG-seq.**

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

Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751

CUT&Tag, CUT&RUN and ChIP-seq assays were performed with HCT116 cells and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751, using the using the CUT&Tag Assay Kit #77552, the CUT&RUN Assay Kit #86652, or the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415 for CUT&Tag samples and DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795 for ChIP-seq and CUT&RUN samples. These tracks compare narrow enrichment at the RPL30 gene, a known target of H3K4me3.

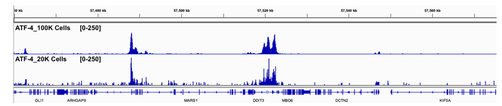
JARID2 (D6M9X) Rabbit mAb #13594



CUT&Tag, CUT&RUN and ChIP-seq assays were performed with NCCIT cells and JARID2 (D6M9X) Rabbit mAb #13594 using the using the CUT&Tag Assay Kit #77552, the CUT&RUN Assay Kit #86652, or the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415 for CUT&Tag samples and DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795 for ChIP-seq and CUT&RUN samples. These tracks compare enrichment around HoxD genes, a known target of JARID2.

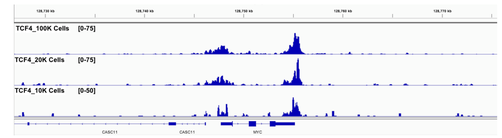
Low Cell Input

ATF-4 (D4B8) Rabbit mAb #11815



CUT&Tag for a transcription factors with only 20,000 cells. CUT&Tag assay was performed with 100,000 or 20,000 Hep G2 cells treated with Thapsigargin #12758 (300nM) for 4h and ATF-4 (D4B8) Rabbit mAb #11815, using the CUT&Tag Assay Kit # 77552. These tracks compare enrichment around the DDIT3/CHOP gene, a known target gene of ATF-4.

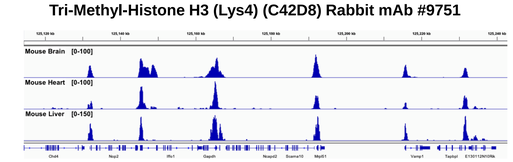
TCF4/TCF7L2 (C48H11) Rabbit mAb #2569



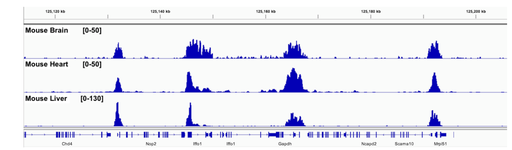
CUT&RUN for a transcription factors with only 10,000 and 20,000 cells. CUT&RUN was performed with 100,000, 20,000, or 10,000 HCT 116 cells (as indicated) using the CUT&RUN Assay Kit #86652. These tracks show binding of TCF4/TCF7L2 at the MYC gene.

Compatible With Tissues

Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751



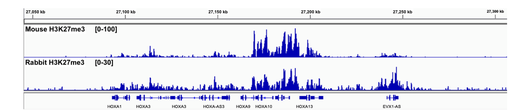
CUT&Tag assay was performed with 1mg fresh mouse brain, heart, or liver tissues and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751, using the CUT&Tag Assay Kit #77552. These tracks show enrichment of H3K4me3 around its known target gene, GAPDH.



CUT&RUN tissue samples may require various fixation conditions and amounts of starting material depending on tissue type. CUT&RUN was performed with 1 mg of lightly-fixed (0.1% formaldehyde, 2 min) mouse brain and liver tissue, 2.5 mg of lightly-fixed (0.1% formaldehyde, 2 min) mouse heart tissue and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751, using the CUT&RUN Assay Kit #86652. These tracks show binding of H3K4me3 around the GAPDH gene.

CUT&Tag with Rabbit and Mouse Primary Abs

Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733



Both rabbit and mouse primary antibodies can be used with the CUT&Tag assay. CUT&Tag was performed with Hela cells and either a mouse H3K27me3 antibody or Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733, using the CUT&Tag Assay Kit # 77552. These tracks compare enrichment around the HoxA genes.

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