Review

# The development and application of cleavage under targets and tagmentation (CUT&Tag) technology

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## Abstract

The regulation of chromatin structure and gene transcription in eukaryotic cells is an involved process mediated by histone modifications, chromatin-binding proteins, and transcription factors. Studying the distribution of histone modifications and transcription factors at the whole-genome level is crucial for understanding the mechanisms of gene transcription. Cleavage under targets and tagmentation (CUT&Tag) is a novel chromatin analysis method that has rapidly gained popularity in the field of epigenetics since its introduction. It has been widely used for the detection of chromatin modifications and transcription factors in different species. Furthermore, CUT&Tag has also been adapted to simultaneously detect multiple epigenetic modifications. Its integration with single-cell transcriptome, and spatial transcriptome analysis allows for a comprehensive examination of cell fate and functions. In this review, we aimed to provide an overview of the recent developments and applications of CUT&Tag and its derivatives, highlighting their significance in advancing our understanding of epigenetic regulation.

**Keywords:** Epigenetic, Cleavage under targets and tagmentation, Histone modification

## 1. INTRODUCTION

Eukaryotic genetic materials are stored in chromatin, a structure that regulates gene expression and controls cellular activities. Chemical modifications of histones can alter nucleosome and chromatin structure while chromatin-binding proteins can directly interact with nucleosomes or DNA to modify the chromatin structure [1,2]. Modulating chromatin accessibility can regulate the binding of transcription factors to DNA, thereby regulating the transcription of genes [3] Therefore, the chromatin structure is crucial for gene transcriptional regulation [4]. With the development of next-generation sequencing technologies, researchers can use chromatin immunoprecipitation techniques (ChIP-seq) to map out the binding of transcription factors and the distribution of histone modifications, advancing the field of epigenetics [5]. However, certain samples, such as primordial cells and those at early embryonic stages, have a limited number of cells, which poses significant challenges for epigenomic studies.

An increasingly favored approach for chromatin analysis is the enzymatic analysis of *in situ* tethering. This approach involves identifying specific chromatin proteins with antibodies or fusion proteins, allowing for the labeling or releasing of the DNA. Over the past 20 years, a variety of techniques have emerged, such as DamID [6], chromatin endogenous cleavage (ChEC) and Chromatin Immuno Cleavage (ChIC) [7]. The *Escherichia coli* Dam methyltransferase was fused with the chromatin protein in DamID of interest, leading to methylation of the DNA near the GATC motif binding site which can

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be cleaved by a GATC-specific restriction endonuclease. Micrococcal nuclease (MNase) cleaves the naked DNA the presence of calcium ions. The MNase is fused with the protein of interest in ChEC or with protein A-MNase in ChIC. Both strategies use the MNase to cleave target protein-related DNA fragments for high throughput sequencing [8].

Compared to ChIP-seq, cleavage under targets and release using nuclease (CUT&RUN) requires as few as 100–500,000 cells, significantly reducing the cost of genome-wide analysis. In addition, CUT&RUN offers base pair resolution of chromatin constituents with background noise significantly reduced [8,9]. Although CUT&RUN is capable of generating high-quality data even from a small number of cells, it requires additional steps such as DNA end repair and adapter ligation for sequencing library preparation. These additional steps increase the overall time and cost. Furthermore, using CUT&RUN to release fragments into the supernatant is not ideal for single-cell platforms. Subsequently, in 2019, Steven Henikoff' laboratory further developed the cleavage under targets and tagmentation (CUT&Tag) technique, replacing MNase with Tn5 transposase (Figure 1) [10]. Because Tn5 carries its own adaptor, there is no need for additional steps like adapter ligation, further reducing the number of cells required for the experiment. This advancement has further promoted the application and research of epigenetics in various fields, such as tissue development, cancer research and neurodegenerative diseases. Since Tn5 acts on DNA through a cut-and-paste pattern and the DNA fragments were not released from the cells, this feature makes Tn5 suitable for single-cell profiling. With continuous technological innovations, single-cell CUT&Tag (scCUT&Tag) has been achieved, allowing for the simultaneous detection of two modifications in the same cell, as well as the simultaneous study of histone modifications and chromatin accessibility in one single cell. Here, we summarized the developments and applications of CUT&Tag in the recent years.

## 2. CUT&TAG

### 2.1. CUT&Tag for bulk cells

CUT&Tag is extensively used for the detection of histone modifications, transcription factors, or RNA Pol II genome-wide. In the earliest version of CUT&Tag, a small number of peaks overlap with the ATAC-seq peaks, which was attributed to the untethered PA-Tn5 binding to open chromatin regions [10]. To overcome this problem, the washing buffer was improved by the addition of 300 mM NaCl to decrease false signals [11]. NPAT, distributed on chromosomes 1 and 6 within the main histone gene cluster, has <100 main peaks, and is virtually not found elsewhere. Therefore, NPAT can serve as a very excellent control to verify the specificity for CUT&Tag. When using chromosome 10 as a reference, there was reportedly significant signal overlap with ATAC-seq peaks at 10 mM NaCl, but it obviously disappeared at 300 mM NaCl. Therefore, 300 mM NaCl solution could effectively eliminate the false signals caused by PA-Tn5.

In addition to the small amount of sample needed, another advantage of CUT&Tag lies in that it requires low sequencing depth and saves costs. In the pioneering studies, CUT&Tag was employed to detect H3K4me2 modification in K562 cells. It was observed that CUT&Tag yielded more peaks even at low sequencing depths, with just 2 million reads compared to 8 million reads required by CUT&RUN [11]. H3K4me3 ChIP-seq in human cells typically requires a sequencing depth of more than 30 million reads, while CUT&Tag can achieve the same peak abundance with only 1 million reads (Table 1). These results demonstrated the high efficiency of the CUT&Tag method.

In addition to its applicability in detecting histone modifications, which are abundant in cells, CUT&Tag is also suitable for studying transcription factors with relatively weaker binding capacity or dynamics. For example, during the differentiation of pluripotent neural progenitor cells into various neural cell types, CUT&Tag was used to analyze the distribution of four key transcription factors: Otx2, Atoh7, Pou4f2, and Isl1. The study discovered that these factors directly bind to enhancers, thereby elucidating the sequence and combination of these factors interacting with the epigenetic environment to control gene expression [12]. Zinc finger protein 143 (ZNF143), belonging to the Krüppel-associated box C2H2-type ZNF family, plays a pivotal role in cell cycle regulation and is intricately linked to the progression of cancer [13]. Study of ZNF143 using CUT&Tag found that the distribution of ZNF143 in HCC and normal hepatocytes was different, suggesting that ZNF143 might serve as a potential therapeutic target for liver diseases [14]. So far, CUT&Tag was widely used for the transcriptional factor analysis in different biological and pathological processes, such as zygotic genome activation [15], spermatogonial stem cells differentiation [16], embryonic mouse forebrain [17], T cells transition during chronic viral infection [18], acute myeloid leukemia [19], Ewing sarcoma [20], breast cancer [21], and lung cancer [22].

Gopalan *et al*. developed an approach called "Multi-CUT&Tag," which utilizes pA-Tn5 to carry adapters with different antibody-specific barcodes. This makes it possible to use different antibodies in the same cell to simultaneously map multiple chromatin-associated proteins or histone modifications [23,24]. The researchers used the method to simultaneously analyze the repression marker (H3K27me3) and activate marker (H3K27ac) in mouse embryonic stem cells. They found that the profiles generated by Multi-CUT&Tag were very similar to those from individual CUT&Tag experiments, demonstrating the reproducibility

and specificity of the method. In addition, as expected, the active histone modification H3K27ac was observed to colocalize with RNAPII. However, they also observed some unexpected co-localization of H3K27ac with repressive marker H3K27me3, suggesting that the method is capable of identifying colocalization of chromatin proteins.

### 2.2. CUT&Tag for single-cell analysis

Although ChIP-seq and CUT&RUN have been utilized for single-cell chromatin analysis, the release of some chromatin fragments into the solution can compromise data accuracy [26-28]. Due to Tn5's ability to bind DNA and add different barcodes to the Tn5 adapters, CUT&Tag is highly suitable for single-cell analysis. Kaya-Okur *et al*. used Takara ICELL8 nano-dispensing system to perform scCUT&Tag for H3K27me3 in K562 cells [10]. Each nanopore is then subjected to polymerase chain reaction with different indexed primers, and finally all DNA libraries in the chip were pooled for in-depth sequencing. The library from each nanopore was distinguished by unique combinations of the two indexes. It is noteworthy that most of the H3K27me3 reads from single cells were included in the bulk sequencing, with high reproducibility between two scCUT&Tag experiments attained [10].

Bartosovic *et al*. further developed the scCUT&Tag protocol by integrating it with 10× Genomics droplet-based single-cell ATAC-seq platform [29]. The oligodendrocyte lineage (OLG) has recently been found to exhibit heterogeneity and change to different cell states throughout development processes. Utilizing scCUT&Tag, researchers were able to resolve cells into discrete populations based on the distribution of histone modifications, identifying unique cell-type-specific epigenetic patterns for unique cell population, and quantitative differences in histone modification levels among different cell populations. With minimal background signals, scCUT&Tag can produce comprehensive genomic tracks from hundreds of cells, achieving a quality comparable to that of bulk ChIP-seq obtained from thousands to millions of cells. In addition, using scCUT&Tag they can map the distribution of H3K4me3 and predict enhancer-promoter interactions. Furthermore, this method is not limited to histone modifications and it also allows for profiling of single-cell binding in profiles for nonhistone proteins, such as the OLG-specific transcription factor and the RAD21 subunit of cohesin complex [29].

For multiplex analysis of chromatin states in one single cell, Bartosovic and Castelo-Branco designed a single-chain secondary antibody, that is, nanobody-Tn5 (nano-Tn5) fusion protein [30,31]. Since nano-Tn5 binds to the primary antibody directly, there is no need to add the secondary antibody from CUT&Tag, thus reducing the times of washes and improving the recovery rate of the nucleus. This nano-CUT&Tag is capable of analyzing small numbers of cells, as little as 25,000, against 150,000 cells with scCUT&Tag protocols [29]. This technique enables simultaneous analysis of three different epigenetic states in a single cell, including two histone markers and chromatin accessibility. In addition, nano-CUT&Tag is able to efficiently process fresh brain tissue samples to generate single-cell resolution multimodal epigenetic profiles. Empolying nano-CUT&Tag, the researchers are now able to simultaneously analyze chromatin accessibility and one or two histone modifications in the brains of young mice, allowing for more detailed differentiation among cell types and states than scCUT&Tag. Researchers have also calculated the chromatin velocity for OLG e translocase access to chromatin (ATAC) and H3K27ac, and deconvoluted the inhibitory status of H3K27me3. Their findings revealed two sequential waves of H3K27me3 affecting various gene modules at different stages of the OLG. With high resolution, adaptability and multimodal capabilities, nano-CUT&Tag provides unparalleled insights into the epigenetic landscape at the single-cell level. However, despite numerous advantages, nano-CUT&Tag does have some limitations. For instance, it has not been extensively validated for the analysis of non-histone proteins (such as transcription factors and chromatin remodelers). In addition, the nano-Tn5 fusion protein is not yet commercially available and requires laboratory purification.

### 2.3. Spatial-CUT&TAG

With the development of relevant technologies, single-cell sequencing can reveal the relationship between one or two histone modifications and gene transcription in a single cell. However, it still lacks spatial information, which is crucial for understanding cell-to-cell communication and interactions, especially in processes such as tumor and tissue development. Spatial transcriptomes can provide the spatial lineage distribution of cells, and researchers are also endeavoring to study the epigenetic characteristics of cells at the spatial scale. This approach promises to bridge the gap between cellular function and the spatial context, thereby enhancing our insights into complex biological systems.

To explore the spatial organization of epigenetic modifications within tissues, a spatial-CUT&Tag technique has been developed. First, an antibody against the target histone modification is added to a fixed tissue section, and then, a secondary antibody is used to enhance the binding of the pA-Tn5. After activation of the pA-Tn5, the adapters containing a linker are inserted into the targeted DNA. Next, a spatial barcode Ai (i = 1 ~ 100) is introduced into the DNA fragments through a microfluidic channel array chip. Then, another microchannel perpendicular to the first flow direction is used to introduce spatial barcodes Bj (j = 1~ 50), attached behind the barcodes Ai. Through

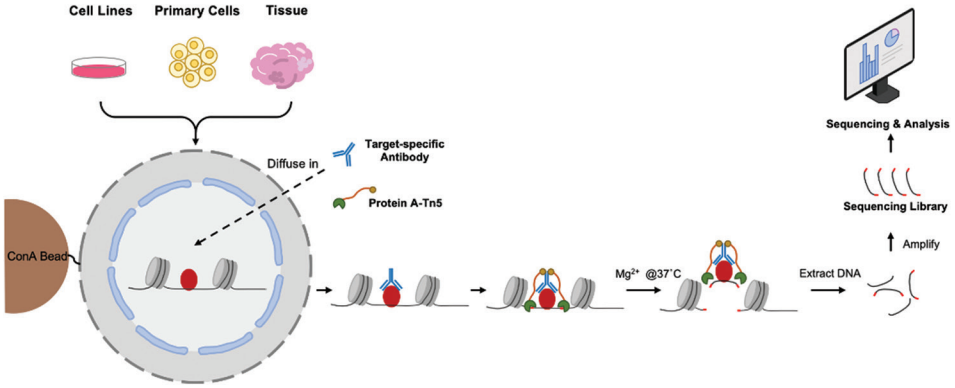


**Figure 1.** The schematic representation of cleavage under targets and tagmentation technology

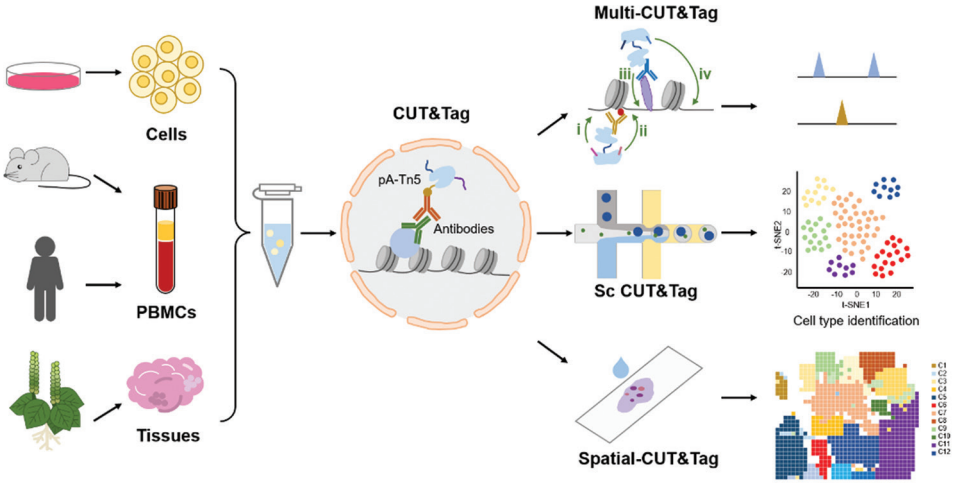


**Figure 2.** The development of cleavage under targets and tagmentation technologies

**Table 1.** Comparison among ChIP-seq, CUT&RUN and CUT&Tag [25]

| Method | ChIP-seq | CUT&RUN | CUT&Tag |
|---|---|---|---|
| Chromatin fragmentation methods | sonication | MNase digestion | Tn5-based tagmentation |
| Number of cells required | ~ 5 million | 100 – 500,000 | 1 – 100,000 |
| Number of reads required | >30 million | 3 – 8 million | 5 – 8 million |
| Singal-to-noise ration | Low | High | High |
| Protocol time (cells to sequencing libraries) | ~ 3 – 5 days | 1 – 2 days | 1 – 2 days |
| Integrated Library preparation? | No, separate library prep required | No, separate library prep required | Yes, direct-to-PCR |
| Ideal targets | Histone PTMs, a wide range of chromatin-associated proteins | Histone PTMs, a wide range of chromatin-associated proteins | Histone PTMs, some transcription factors |
| Automation compatibly | Low | High | High |

ChIP-seq; Chromatin immunoprecipitation techniques; CUT&RUN: Cleavage under targets and release using nuclease; CUT&Tag: Cleavage under targets and tagmentation; PCR: Polymerase chain reaction.

the combination of barcodes Ai and Bj, a two-dimensional information can be defined. The tissue section is imaged after these steps to correlate the tissue morphology with the spatial epigenomic map. Tissue slices were then imaged to correlate tissue morphology with spatial epigenomic map. Finally, double-labeled genomic DNA fragments were released from the genome DNA for library sequencing. In order to facilitate the analysis of the data, the authors developed a pre-processing pipeline and shared it with other researchers online [32].

Using spatial-CUT&Tag, the researchers obtained 10,064 (H3K27me3), 7310 (H3K4me3), or 13,171 (H3K27ac) unique fragments from mouse embryonic day 11 (E11) embryos at a 20-μm pixel size. By comparison with published mouse brain scCUT&Tag, it was found that spatial-CUT&Tag detected

more unique fragments than scCUT&Tag under the same sequencing depth conditions. However, spatial-Cut&Tag had lower fraction of reads in peaks than scCUT&Tag, possibly due to the use of frozen samples. In addition, combining spatial-CUT&Tag with either scRNA-seq or scCUT&Tag makes it possible to predict the region where specific cell types are located [32]. Zhang *et al*. used Spatical CUT&Tag-RNA-seq to simultaneously detect the spatial information of histone modification distribution and transcriptome. This technology possesses distinctive advantages in spatial analysis. For example, H3K27me3 scCUT&Tag analysis alone cannot clearly identify several cell clusters, but it can be further distinguished by scRNA-seq data from the same slices. This suggests that combining spatial-CUT&Tag with scRNA-seq (spatial Cut&tag-RNA-seq) can more accurately identify cell clusters or states [33]. The integrated analysis of spatial epigenetics and transcriptomics can unveil gene regulatory alterations specific to cell types in disease states, which is especially valuable for the study of neurodegenerative diseases, cancer, and other diseases influenced by cell location (Figure 2).

## 3. CONCLUSION

With the advent of second-generation high-throughput sequencing technology, epigenetic research has been developing rapidly. This advancement enables scientists to explore chromatin structure and gene expression from multiple perspectives, such as chromatin accessibility [34], nucleosome positioning [35], histone modifications, transcription factors, and chromatin-binding proteins. Studying various epigenetic modifications and chromatin states on a genome-wide scale helps us to understand the molecular mechanisms underlying various cellular activities. However, traditional ChIP-seq technology has certain limitations, such as high cell number requirements and high noise due to chromatin sonication, restricting its use for many biological samples, including embryonic development and patient samples.

The emergence of CUT&Tag technology has effectively solved the limitations of traditional ChIP-seq. First, the activity of the PA-Tn5 transposase eliminates the need for complex procedures such as end repair and adapter ligation. This innovation significantly minimizes DNA loss, making it possible to analyze samples with low cell numbers. Second, CUT&Tag generates data with a high signal-to-noise ratio, reducing the required sequencing depth and associated costs [11]. Third, as the entire process can be completed within a 2-day using standard laboratory equipment, this method does not entail specialized ultrasonic disruption devices, making it accessible and efficient for laboratory application. Fourth, Tn5 binds to its adaptors, and therefore it is suitable for single-cell research. When combined with single-cell RNA-seq, single-cell CUT&Tag can accurately identify the relationship between chromatin structure and gene transcription regulation at the single-cell level.

As CUT&Tag technology continues to improve, its combination with other methods will significantly push epigenetic research forward.

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## CONFLICT OF INTEREST

The authors have no competing interest to declare.

## AUTHOR CONTRIBUTIONS

*Conceptualization:* Guohong Li, Xi Wang
*Visualization:* Xinglin Li
*Writing – original draft:* Chaoyang Xiong, Jiyin Wang
*Writing – review & editing:* All authors.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## DATA AVAILABILITY

Not applicable.

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