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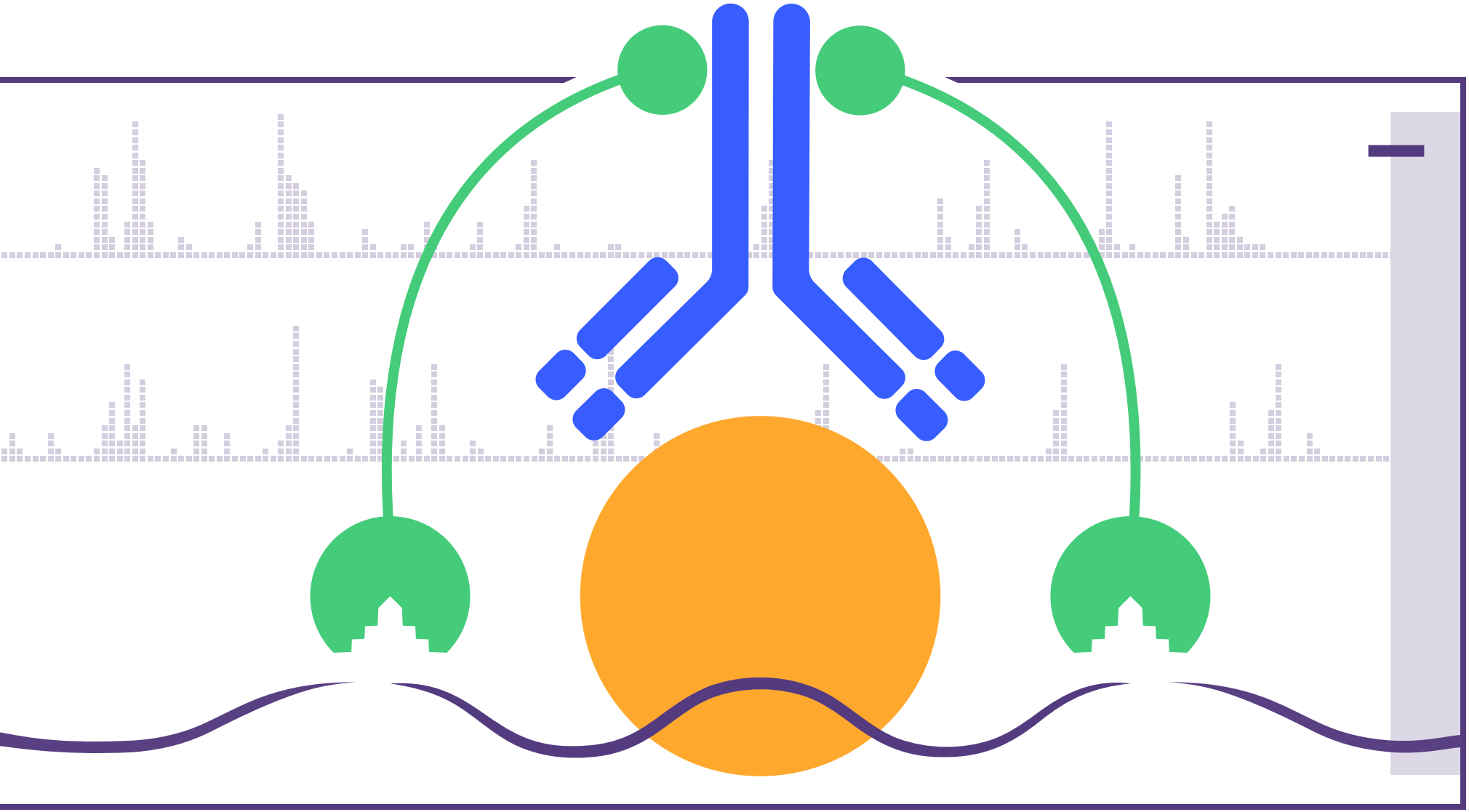
Enabling Epigenetics Research

CUT&RUN Complete Guide

Overview, Method, and Resources

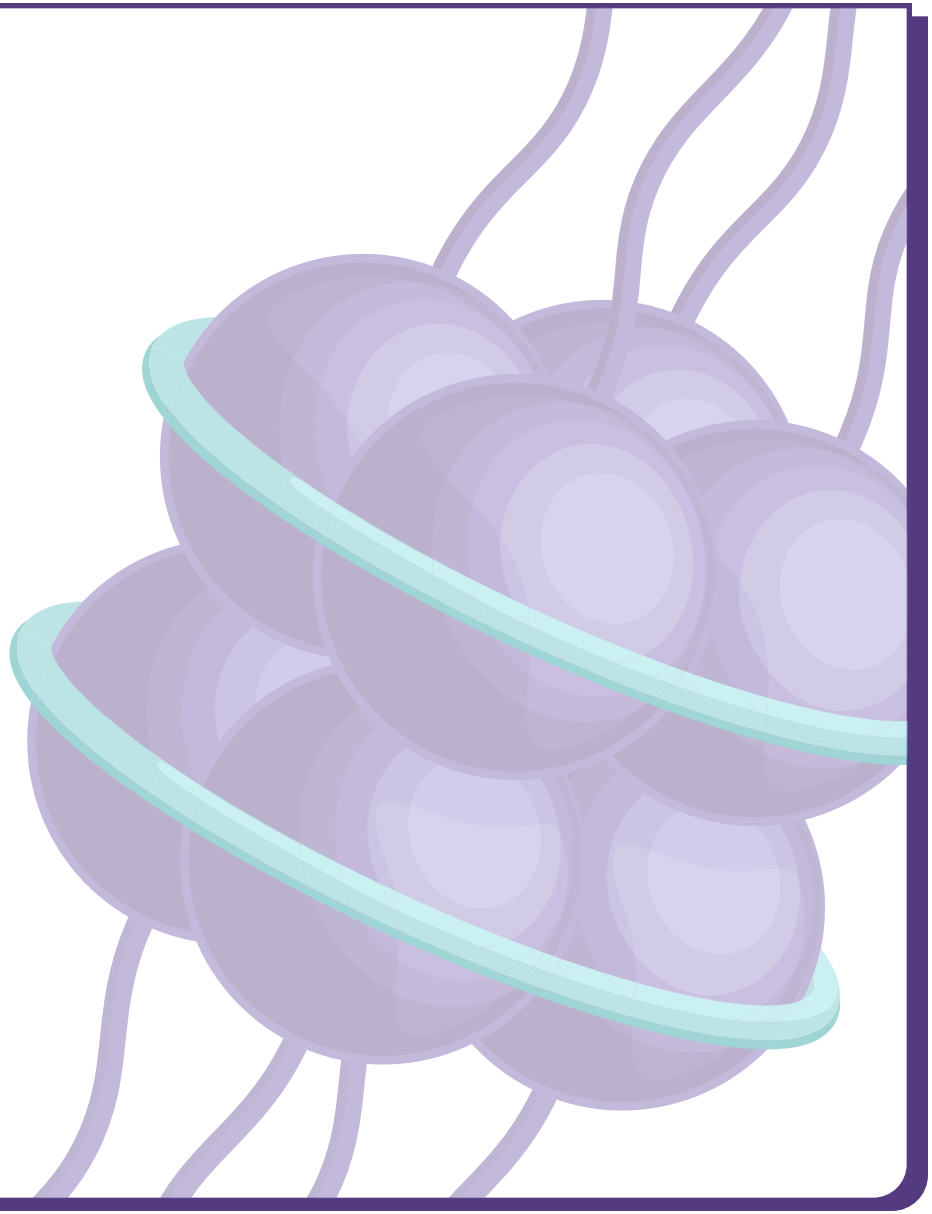


Introduction

CUT&RUN (Cleavage Under Targets & Release Using Nuclease) is an epigenetic method used to investigate the genome-wide distribution of various chromatin-associated proteins and their modifications. CUT&RUN is a derivative of chromatin immunocleavage (ChIC). CUT&RUN is similar to chromatin immunoprecipitation (ChIP), in that it utilizes an antibody to target chromatin-associated marks and proteins but requires less sample material and less sequencing depths than ChIP.

CUT&RUN is a valuable tool for studying chromatin-associated proteins because it is sensitive, specific, and requires fewer cells than ChIP, making it ideal for identifying binding patterns of chromatin-associated proteins such as transcription factors or histone modifications genome-wide. Chromatin-associated proteins play critical roles in regulating various cellular processes such as gene expression, DNA replication, DNA repair, and cell differentiation. Understanding the binding patterns of these proteins can provide insight into how these cellular processes are regulated.

With this eBook, you will find useful information – from the background and novelty of the CUT&RUN method, to evaluating when it's the best choice for your research, followed by sample data, analysis tips, and resources for further study.



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Overview of CUT&RUN

Chromatin immunoprecipitation is the gold standard technique for analyzing chromatin state as well as transcription factors, cofactors, and histones binding to DNA. It is a multi-step process where each step is of crucial importance to obtain relevant and reproducible results. Moreover, it needs a significant amount of cross-linked material. Although there have been significant improvements in sequencing technologies over the last few decades, for ChIP the procedure has remained essentially the same. Despite its utility, ChIP has a number of limitations such as cell number and the need for fixation of the sample material. A number of alternative methods have been developed in recent years to address these limitations. Two such methods are **CUT&Tag** (Cleavage Under Targets and Tagmentation) and **CUT&RUN** (Cleavage Under Targets and Release Using Nuclease), developed in the lab of Steven Henikoff in the Fred Hutchinson Cancer Center at University of Washington.

These new methods have their own unique limitations, and this eBook will highlight the Pros and Cons of one of these new techniques, CUT&RUN.

CUT&RUN is used for the same purpose as cross-linked ChIP-Seq (XChIP-Seq), i.e., mapping DNA-protein interactions. CUT&RUN is an *in situ* assay using native/non-fixed cells or isolated nuclei. It is based on the ability of micrococcal nuclease (MNase) to cut chromatin and uses a chimeric fusion protein, pAG-MNase (pAG-Mn). Protein A binds specifically to the Fc fragment of Immunoglobulin and leads the MNase digestion at the vicinity of the antibody. It consists of 4 steps: immobilization of the cells, incubation with antibodies, digestion of DNA, and library preparation. The native cells are immobilized on magnetic Concanavalin A beads which takes advantage of the ability of Concanavalin A to bind to transmembrane proteins. After that, cells are permeabilized. Immobilization allows the cells to go through a number of wash steps while minimizing loss of materials. Primary and secondary antibody incubations are now performed after which the pAG-Mn protein is added with Ca^{2+} to activate the enzymatic reaction. Protein A binds to the secondary antibody and the MNase cuts DNA on both sides of the protein of interest. Protein/digested DNA complexes are released through the pores of the permeabilized cell. After the purification of DNA, the sequencing library is prepared and is ready to be sequenced.

In Steven Henikoff's original article, this protocol was successfully tested in yeast and humans for abundant or low-expressed transcription factors. The data was comparable to cross-linked ChIP-Seq data with better resolution and fewer sequencing reads. In this publication, 500,000 yeast nuclei and 10 million Human K562 cells were used, but S. Hainer *et al.* were able to perform the procedure with as few as 5000 cells.

CUT&RUN vs. CUT&Tag 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 (Next Generation Sequencing) 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 nuclei isolated from live cells that are immobilized on Con A 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&RUN, like ChIP-Seq and CUT&Tag, allows the identification of protein/DNA binding sites. It is a sensitive method that uses a secondary antibody as an anchor for the MNase to cleave DNA at the binding site of the protein. The low background noise makes this technique suitable for working with low-cell numbers, as well as for transcription factors (CUT&Tag has the edge for histone marks). Moreover, cost-effectiveness and a straightforward protocol make this method ideal for high-throughput assays. CUT&RUN is a new technique to add to the epigenetic tool box. Its sensitivity, cost effectiveness, and relative ease of use bring epigenetics to a whole new level and promises exciting discoveries in the field.

	CUT&RUN	CUT&Tag	ChIP-Seq
Performed under native conditions	Yes	Yes	No
Chromatin fragmentation method	MNase digestion	Tn5-based tagmentation	Sonication
Cell number requirements	500,000 cells	5,000-500,000 cells	1-10 million cells
Sequencing depth required*	8 million reads	2-8 million reads**	20-50 million reads†
Integrated library preparation	No	Yes	No
Compatible targets	Histone modifications, transcription factors, and co-factors	Primarily histone modifications, some transcription factors, and co-factors	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, 8-10 million reads are recommended
†25 million reads are recommended for transcription factor targets

Advantages of CUT&RUN

CUT&RUN has several important advantages over XChIP, which include requirements for a lower cell number, shorter protocol, less sequencing depth, and better resolution. The typical XChIP-Seq protocol starts with cross-linking and chromatin shearing (enzymatic or mechanical), followed by antibody binding, and purification with protein A. After decrosslinking, the library is prepared for NGS. It involves many steps and usually requires millions of cells as starting material.

CUT&RUN can be performed with as few as 5000 cells or nuclei. Fixation is not required which can be an advantage as paraformaldehyde can cause artifacts and antigen masking.

Whereas in XChIP, chromatin is randomly sheared in 350bp fragments, in CUT&RUN, the pA-MNase cuts directly in the vicinity of the antibody. Only small fragments of interest are released by the pores of the cells and sequenced. That's why the resolution of the CUT&RUN is better than XChIP-Seq with less material. CUT&RUN does not require fixation, shearing, or decrosslinking. Overall, the procedure is shorter and results in a more accurate protein/DNA mapping. CUT&RUN is also more cost-effective because of the low starting material, lower antibody requirement, and less deep sequencing. Although the advantages of CUT&RUN are very appealing it does have certain limitations.

Limitations of CUT&RUN

CUT&RUN, as with every molecular biology technique has limitations. The majority of limitations are due to the use of native starting material. Native cells or nuclei must be immobilized on Concanavalin A beads. The membrane structure needs to be intact to ensure good binding to the beads. Cells can't be snap-frozen, but can be cryopreserved. If analyzing tissue, the best solution is to isolate nuclei, which can be long and complicated depending on the type of tissue. It also renders the analysis of factors with low affinity to DNA almost impossible because the interaction is lost during the different washing steps.

The other challenge is the antibody used in the procedure. ChIP-Seq-validated antibodies are not automatically suitable for CUT&RUN. Henikoff recommends testing antibodies validated in immunofluorescence because buffer compositions are similar. As a result, antibody providers have started validating more and more antibodies for CUT&RUN and CUT&Tag.

Finally, CUT&RUN is still a recent technology, and comparing CUT&RUN data to previous XChIP-Seq data remains the best way to validate the experiment.

Discoveries Enabled by CUT&RUN

Developmental Biology

Gehring's lab at MIT adapted the CUT&RUN protocol to analyze the development of *Arabidopsis* endosperm. Epigenetic regulation is an important mechanism in endosperm and seed development, particularly PRC2-regulated H3K27me3. Each seed only contains a few hundred endosperm nuclei, however, ChIP-Seq analysis requires hundreds of thousands of cells. They previously used INTACT, which is a technique to isolate tagged nuclei in specific cell types. INTACT ChIP-Seq requires 500,000 nuclei. They were able to perform CUT&RUN using 50,000 endosperm nuclei and 5 million sequencing reads to profile H3K27me3. They obtained 65% overlapping peaks with INTACT ChIP-Seq. They then performed CUT&RUN using leaf nuclei, and again obtained a very similar H3K27me3 profile to ChIP-Seq data.

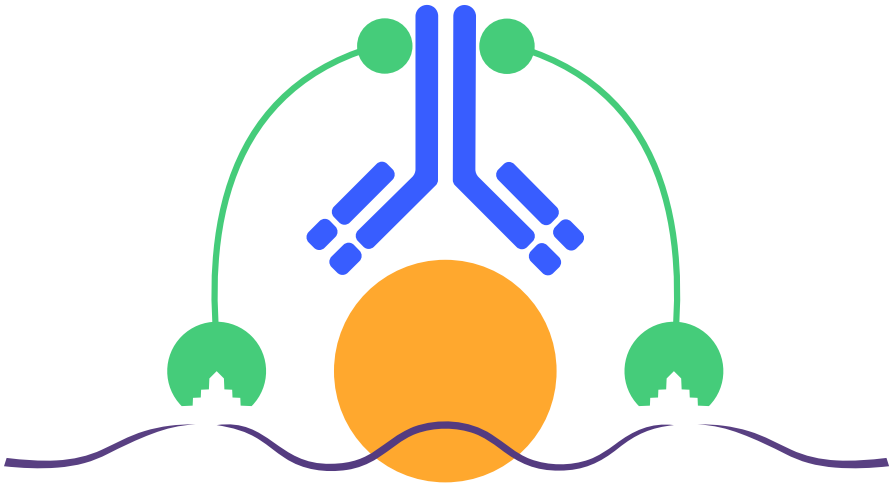
Hainer's lab at the University of Pittsburgh, went even further by adapting CUT&RUN starting with a single cell to analyze the protein/DNA binding profile in blastocytes (uliCUT&RUN). They developed the protocol using a single blastocyst, a small population of cells, or a single cell, focusing on reducing background and increasing output. They obtained qualitative profiles for transcription factors, allowing the study of molecular mechanisms in rare samples.

Cancer Research

Considering the importance of epigenetic deregulation in tumorigenesis as well as the large amount of patient samples, it is no surprise that CUT&RUN has been used in this context.

Acute Myeloid Leukemia is a frequent childhood cancer. Tumor cells bearing NUP98-NSD1 and MLL-r chromosomal abnormalities produce oncogenic chimeric proteins that activate a set of stemness and proliferative-related genes leading to patients' poor outcomes. In a recent publication by Ren *et al.*, mouse NUP98-NSD1 AML cells were used to analyze PRC2 and Kdm5b (lysine demethylase) antagonistic behavior in AML tumorigenesis. Integrating RNA-Seq, ChIP-Seq, and CUT&RUN, Wang's team at Chapel Hill was able to demonstrate that Kdm5b directly binds to DNA and represses stemness genes. Altogether, they demonstrated that the PRC2-Kdm5b axis is a key regulator of AML oncogenesis.

Melanoma has been found to display the ability to transition from proliferative state to invasive state through reversible epigenetic mechanisms. The epigenetics regulators, in particular bromodomain proteins, are known to be involved in melanoma invasive properties. Espinosa's lab at the Center for Molecular Medicine, Karolinska Institute, focused on TRIM28 and showed that it promotes the metastatic state through YAP signaling and slows down proliferation through RAS inhibition. TRIM28 controls the switch between proliferation and invasion by inhibiting JUNB transcription. Integrating TRIM28 interactome, RNA-Seq, CUT&RUN, and ChIP-Seq, Nyberg *et al.* discovered that the TRIM28-JUNB axis regulates the switch between proliferation and invasion in melanoma.

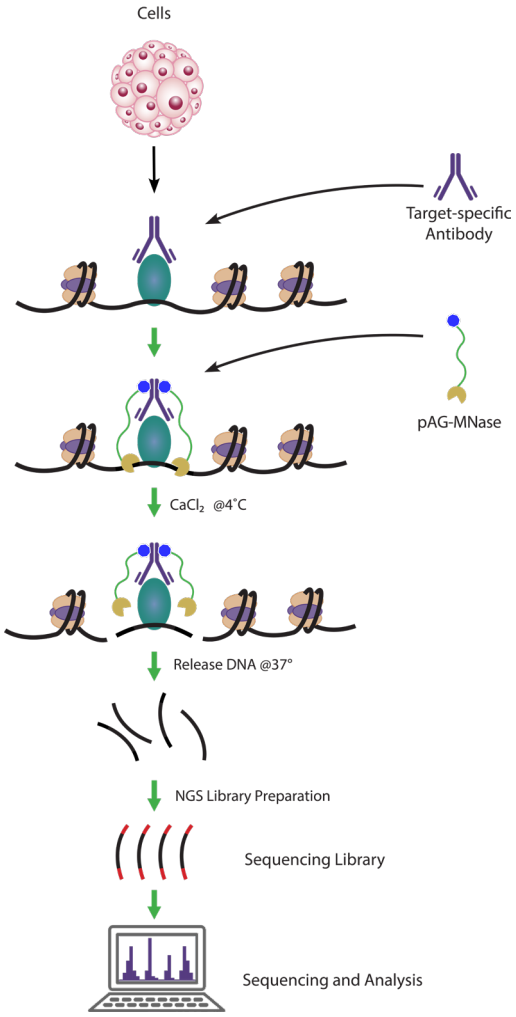


CUT&RUN Method

Workflow

In CUT&RUN, a protein of interest is tagged with an antibody and bound to the chromatin in intact cells. Then, a micrococcal nuclease (MNase) is used to cleave the DNA specifically at the binding sites of the protein of interest. The released fragments are purified, sequenced, and mapped to the reference genome to determine the protein's binding sites. Unlike ChIP, CUT&RUN does not require crosslinking of the protein to the DNA, which can introduce artifacts.

CUT&RUN is a valuable tool for studying chromatin-associated proteins because it is sensitive, specific, and requires fewer cells than ChIP, making it ideal for identifying binding patterns of chromatin-associated proteins such as transcription factors or histone modifications genome-wide. Chromatin-associated proteins play critical roles in regulating various cellular processes such as gene expression, DNA replication, DNA repair, and cell differentiation. Understanding the binding patterns of these proteins can provide insight into how these cellular processes are regulated.



References:

1. Schmid, M. *et al.* (2004) *Mol. Cell*, 16(1): 147-157
2. Skene, P.J. *et al.* (2017) *Elife 6*, e21856
3. Skene, P.J. *et al.* (2018) *Nat Protoc.*, 13, 1006-1019

Sample Data

Histone Mark Profiling with ChIP-Seq, CUT&Tag, and CUT&RUN

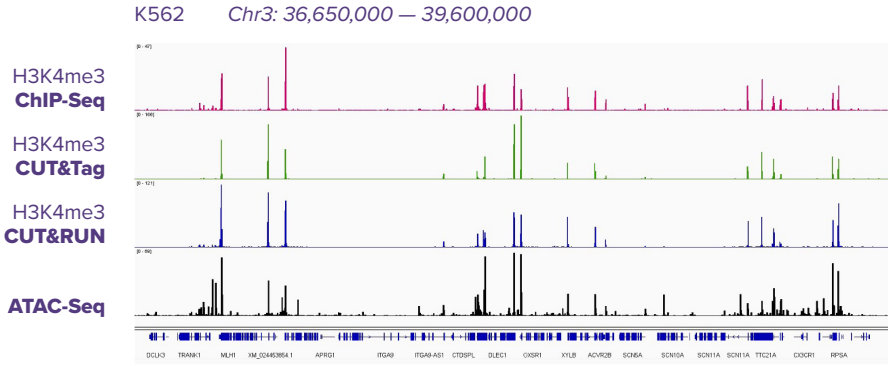


Figure 1. Comparison of Methods in K562 cells, Using H3K4me3 Antibody

Genome browser tracks of ChIP-seq, CUT&Tag and CUT&RUN are compared to show similar enrichment profiles. CUT&RUN and CUT&Tag have slightly lower background than ChIP-Seq. The ChIP-Seq track was generated with 1M K562 cells, CUT&Tag with 100K, and CUT&RUN with 500K cells. ATAC-Seq specific peaks are shown (50K cells), demonstrating that free pA-Tn5 does not contribute to signal generated in CUT&Tag.

Method Selection Guide

Use our selection guide when considering which methods best suit your research and sample type.

Sample Type	Cultured Cells			Tissue
Profiling Protein of Interest	TFs or Histones	TFs	Histones	Histones
Input Quantity Required	5-500K	50-100K	5-100K	0.5-10mg
Most Important Feature	Targeted MNase Cleavage	Low Input	Integrated Library Prep Step	Integrated Library Prep Step
Sequencing Depth Required	8-25 Million Reads	20-50 Million Reads	8-10 Million Reads	8-10 Million Reads
Recommended Kit	CUT&RUN #53180	Low Cell ChIP #53086	CUT&Tag-IT #53160	CUT&Tag-IT #53170

Peak Analysis – a Discussion with Dr. Claudio Cantu

A challenging question, and subject of lively debate, it how to interpret peaks in methods such as ChIP, CUT&Tag and CUT&RUN. In a recent episode of the Active Motif Epigenetics Podcast, we talked to Claudio Cantù from Linköping University about his work on peak blacklists, peak concordance and the burning question: **what is a peak in CUT&RUN?**

In the podcast, Claudio Cantù share's his lab's work to define a set of suspicious peaks that can be used as a "peak blacklist" and can be subtracted to clean up CUT&RUN data sets. The lab also worked on a method called ICEBERG (Increased Capture of Enrichment By Exhaustive Replicate aGgregation) to help define peaks from a number of experimental replicates. By using this algorithm, the team is trying to discover the beta-catenin binding profile, not the tip of the beta-catenin binding iceberg, but the whole of the beta-catenin binding profile. We present some highlights from the podcast here.

Question: The ENCODE project and also others have compiled a blacklist for ChIP-Seq which have been widely adopted. These lists contain regions of high and unstructured signal regardless of cell types or protein target, and you set out to do the same for CUT&RUN. What was the initial situation you found yourself in when starting with this?

Dr. Cantu: Every time we do a CUT&RUN experiment, we have been using the previous blacklist. That is used for ChIP, because the reasoning is that those regions, the regions that are present in a black list, have an obscure origin in the sense that many are recognizable as regions of the genome that are difficult to be mapped in principle, because they are highly repetitive and they can be subject, for example, to also mapping artifacts. You can have a few duplicated regions or repetitive regions, and then many of the amplified fragments in your sequencing library map there maybe as an artifact and so on. Then, what you see, as a matter of fact, is a signal that looks like a peak, but then you would see this signal also when you do a non-antibody experiment or an IgG control or a non-related antibody targeting something that is not related to your biologic hypothesis. You would see a signal there.

Then, very wisely, I believe that someone had the idea to compile a list of regions that it's good to discard, because they show signal … Again, the ChIP-Seq blacklist might have included many types of artifacts, that are mapping artifacts, that I suggested before, but they could also have been, for example, cross-linking artifacts. We cannot exclude that when you apply cross-linking, there are maybe big protein complexes somewhere in the genome that have nothing to do with the protein that you are trying to pull down, yet they get cross-linked and for some chemical reason they are preferentially or they are enriched also in your pull-down, so you find signal there. Many are the reasons why you get spurious

signal, and they were very wise in compiling this ChIP-Seq blacklist, and we have used those datasets to subtract it from our datasets.

Then we realized that ... Then, we have many projects in the lab that essentially are CUT&RUN centered, and we realized that many of those regions that are spurious signal and identified in ChIP-Seq blacklist are also appearing in the CUT&RUN experiment, so we thought it's wise to remove them. Then in particular, Anna Nordine, now, she's a PhD student in my lab, she realized that there were other regions that appeared often in our CUT&RUN experiments, but they were not included in the CUT&RUN, sorry, in the ChIP-Seq ENCODE blacklist. I have to admit, entirely on her initiative, she compiled a list, and she proposed this project to me. Of course, I was very enthusiastic because of the importance of the project. I mean, we all need to rely on the good tool made by ENCODE, which however, for CUT&RUN it's not complete, because CUT&RUN identifies new regions that are also found when we use an IgG sort of [inaudible 00:18:26] serum, like a mixture of annulated antibody, as a control. We thought it's important to tell the community that those regions should raise suspicion at least.

Question: You took datasets from your own lab and then also from other labs and you bioinformatically compiled them together and made an average of all the peaks that might come up in all of them?

Dr. Cantu: This was precisely what we have done, in a nutshell. Originally, historically in the lab, we had done several IgG controls, and we started compiling a black list using those, and we noticed that if we take 20 IgG controls that we have done and looked at all the peaks that are called, let's say for example, in a majority of the experiments, then we would have a list of suspect regions that appeared in our own experiments. If we used, let's say, call it our lab blacklist, and when we applied this to other datasets, we could see that those datasets were improved in the sense that most of the datasets also included those signals, which then ... Of course, we always rely on the fact of specificity, so we trust the peculiarity of our discovery when the signal is not found in the control. In the very moment in which we noticed that across the board of our experiments targeting our favorite transcription factors, the signal that we had identified in the blacklist that we had compiled was present, then we felt the urge of excluding it. This might be a genuinely good signal, but because it appears also in the control, it's safer to exclude it, so we want to remove false positives.

Question: How does the CUT&RUN blacklist compare to the ENCODE blacklist? Is there something that's come up that is maybe interesting, or is it just a list of peaks that does not tell anything more interesting than just using it?

Dr. Cantu: I would have to go into the wild world of speculation here, because there are peaks overlapping with the ENCODE blacklist, but not all of them. The CUT&RUN blacklist doesn't identify some of the ENCODE blacklist, and the CUT&RUN blacklist is smaller. It's a smaller set of peaks, and many are in regions that are obviously, when you look at the sequence, and you are in a centromere and then sequence, there are a lot of repetitive regions and there are a lot of peaks there that appear across our IgG experiments and across the counter-experiments of the others, other published datasets from independent laboratories. Those sequences don't tell me much, but of course, I am a person with limited ability to see through DNA sequencing regulatory regions. Some of those regions, as I said, I believe we have for example one blacklist or suspect list peak in the GSK3 promoter, which is of course a very important gene. Then we get this peak across our experiments, and we have to exclude it because it's present in the blacklist.

However, we wish to warn caution in the sense that someone is bound to identify a factor which regulates GSK3, which is a gene that must be GE must be regulated on the promoter by transcription

regulators. Also, so this is why I favor also the change of name from blacklist to suspect list, because I think that scientists also have to use those lists with [foreign language 00:25:51], a little bit of caution, and look at those instances individually in each of your experiments.

Question: It would be interesting to see if there is a regulation that the promoter does this blacklist peak change indeed, right? I mean, if it's still the same after there is indeed a regulation, but it still shows up, it might be dangerous for the interpretation of this specific experiment.

Dr. Cantu: We have looked at the motifs, the transcription factor binding motifs of the regions that we identified in the blacklist, and there are quite a lot of things going on. You can think, for example, we identified CTCF motifs, and then you can think, well, is it CTCF binding that produces spurious signal and so on? It doesn't look like the case when we, for example, overlap the blacklist with our CTCF binding profile. Also, when we looked at that, we have done the same analysis of motif search in the ENCODE blacklist, and also there we identified quite a lot of transcription factor binding signatures.

I don't think that we can learn much from this, for the reason that motifs are degenerated DNA sequences, so a motif could be a bad motif and does not really allow stringent binding of transcription factors. Also, motifs are short DNA sequencing, so the more you expand the pool of the DNA sequencing you are screening, the higher the chance of identifying by mere luck some motifs. I don't think that you necessarily learn something from those. The ultimate test would be, is that region actually bound by that transcription factor with that motif? Then we need to do a ChIP-Seq or a CUT&RUN.

Question: How many peaks are overlapping with ChIP-Seq experiments and how is the overlap of peaks and biological replicates in CUT&RUN? To solve this you developed ICEBERG, when is a peak really a peak?

Dr. Cantu: This is a million-dollar question, whose answer, in my opinion, is no one knows. No one really knows, and what we have to do ... The reason why I say this is because the peak is identification of signal in specific genomic coordinates. Now, as it turns out, for example, when you do ChIP, in ChIP you are purifying cross-linked genomes, and then you hope that via immuno-precipitation, you are enriching for those DNA regions that are bound by your transcription factor. Enrichment precisely means that there will be a ratio between the signal in that position and the left and right adjacent sites.

This is the signal to noise ratio, which you need. This needs to be higher than one, in the sense that if the signal to noise ratio is one, in that position, your signal is equivalent to background, so you cannot draw any conclusion. Is the signal to noise ratio 1.1, 1.2, 1.8, 2.5? What is the number where we trust it as a signal, as long as it is reproducible? This is a genuinely difficult question that pertains to the signal detection theory, of which I cannot say more in terms of math, but I only have an intuitive understanding of it. It's a problem of detecting anything. Even if you are driving, piloting a plane, and you have a radar and you spot a signal, this might be another fighter jet trying to bombard us, or maybe it's a flock of birds. Then you need to distinguish these two signals, and this is a genuine problem in all fields of human enterprise where we need to detect something that is not something else.

For further conversation on how to determine how many replicates are enough, what is the new ICEBERG method, and how to apply this analysis to single-cell methods, listen to the full podcast here.

Reagents for CUT&RUN

CUT&RUN Assay Kit

The Active Motif ChIC/CUT&RUN Assay Kit is a valuable tool for studying chromatin-associated proteins because it is sensitive, specific, and requires fewer cells than ChIP, making it ideal for identifying binding patterns of chromatin-associated proteins such as transcription factors or histone modifications genome-wide.

Product	Format	Cat. No.
ChIC/CUT&RUN Assay Kit	24 rxns	53180
ChIC/CUT&RUN pAG-MNase	50 rxns	53181

CUT&RUN Assay Kit Features

- Compatible with 5,000 to 500,000 cells
- Complete kit with optimized protocol
- Developed for genome-wide chromatin-associated protein profiling

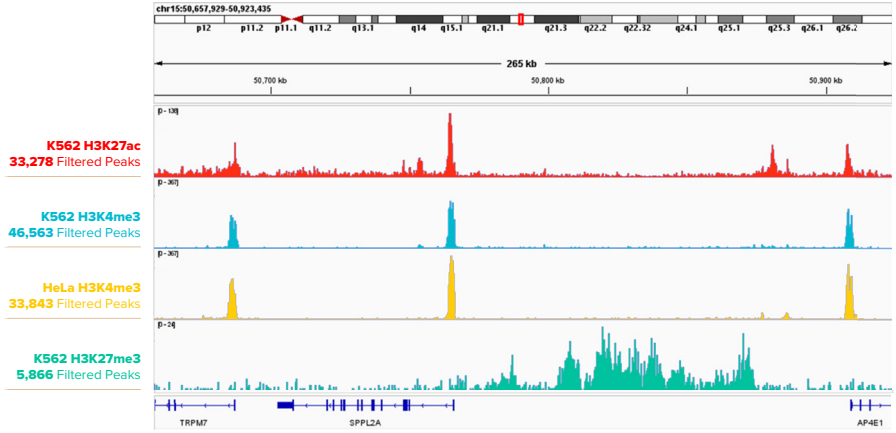


Figure 2. CUT&RUN Profiling Histone Marks in K562.

IGB browser tracks are shown for 500,000 K562 cell CUT&RUN peaks with Active Motif antibodies H3K27ac (Cat. No. 91193), H3K4me3 (Cat. No. 91263) and H3K27me3 (Cat. No. 39155).

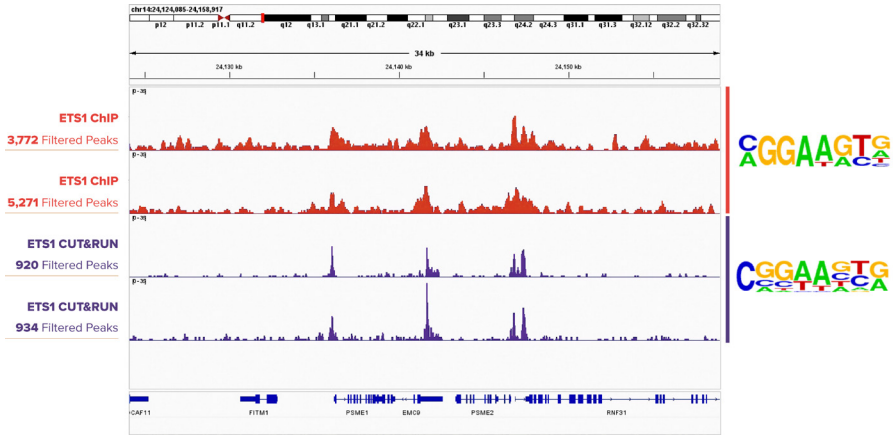


Figure 3. ETS1 Peak Profiles in CUT&RUN Compared to ETS1 ChIP-Seq Peak Profiles.

IGB browser tracks for ChIP-Seq (top two tracks) in DIPTG cells and CUT&RUN (bottom two tracks) in K562 cells assays targeting ETS1 (Cat. No. 39580). Sequence logos from Homer analysis (to the right of the tracks) show motif enrichment for the ETS1 motif in both sample sets.

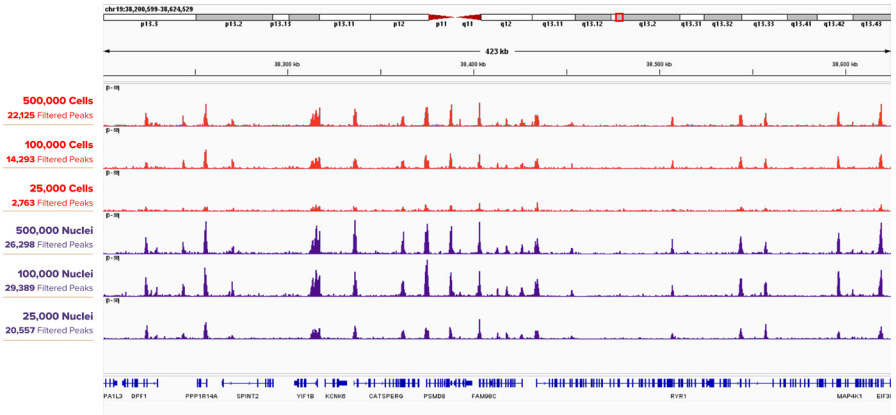


Figure 4. CUT&RUN H3K4me3 Assay Results Comparing Titrations of K562 Cells versus Nuclei.

500,000, 100,000, 25,000 K562 Cells (top three tracks) or Nuclei (bottom three tracks) were assayed in CUT&RUN with Active Motif's H3K4me3 antibody (Cat. No. 39060). Filtered Peaks remain higher at lower input for nuclei than cells, and the browser tracks for nuclei show stronger peaks for each input amount when compared to cells.

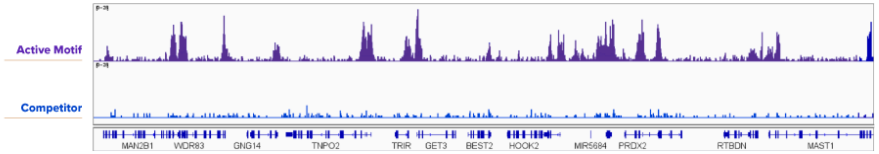


Figure 5. Active Motif's CUT&RUN Assay Kit is Compatible with 5,000 Nuclei for Histone Marks.

5,000 fresh K562 nuclei were assayed using Active Motif's CUT&RUN Assay Kit and Competitor CUT&RUN Assay Kits using Active Motif's H3K4me3 antibody (Cat. No. 91263). Active Motif's CUT&RUN Assay Kit yielded more robust results than the competition.

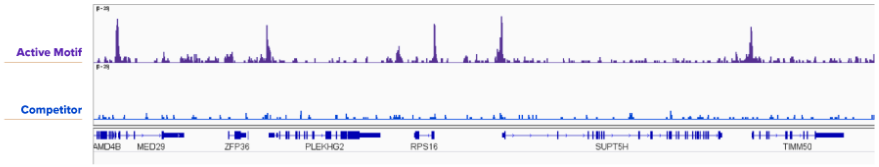


Figure 6. Active Motif's CUT&RUN Assay Kit is Compatible with 25,000 Nuclei for Transcription Factors.

25,000 fresh K562 nuclei were assayed using Active Motif's CUT&RUN Assay Kit and Competitor CUT&RUN Assay Kits using Active Motif's YY1 antibody (Cat. No. 61980). Active Motif's CUT&RUN Assay Kit yielded more robust results than the competition.

500K nuclei, 100K nuclei, 25K nuclei, and 5K nuclei

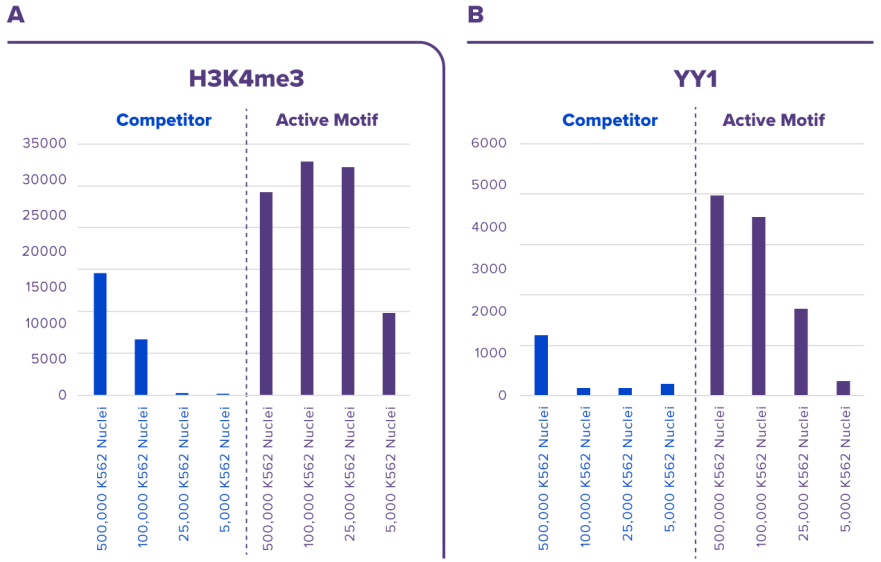


Figure 7. Active Motif's CUT&RUN Assay Kit Outperforms the Competition.

500,000, 100,000, 25,000, and 5,000 K562 nuclei were assayed in CUT&RUN Kits from Active Motif and Competitor for YY1 (Cat. No. 61980) and H3K4me3 (Cat. No. 39159).

See our CUT&RUN Assay Kit FAQs for more data and examples of results with cells versus nuclei.

CUT&RUN 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&RUN assays. View the full list at activemotif.com/cut-run-ab

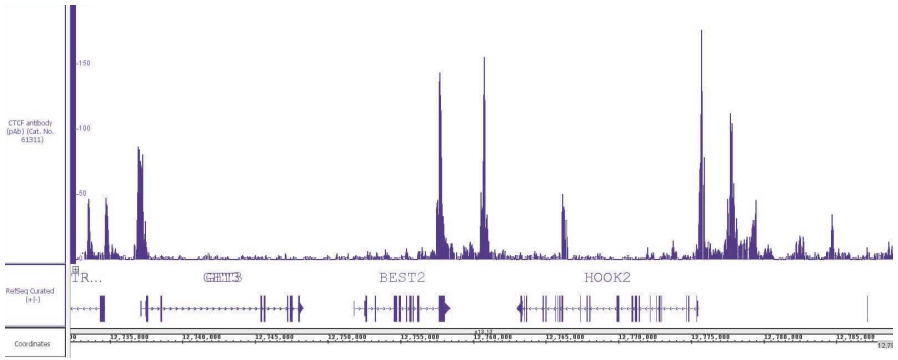


Figure 7. CTCF antibody (pAb) tested by CUT&RUN

CUT&RUN was performed using 500,000 K562 cells and sequenced using 38 base-pair, paired-end reads on the Illumina NovaSeq. Data was collected from 32 million reads, and CTCF data is show for Chromosome 19. (Cat. No. 61311)

Antibodies Published for Use with CUT&RUN

Active Motif maintains a curated list of products and services published by our customers for use in their research.

Our ever-expanding list of antibodies published for CUT&RUN may be found at activemotif.com/publications

Supplementary Materials

FAQs

Question	Answer
The nuclei pellet is not visible, is it there?	The pellet may not be visible. Be sure to position tubes with caps oriented in centrifuge so that you know where the pellet should be in the tube, and proceed carefully with the next steps.
What is the recommended sequencing path?	25 million reads for transcription factors. For some histone marks you may go down to 2 million reads.
How many cells should I use for transcription factor targets?	We recommend 500,000 cells and a CUT&RUN validated antibody.
Why is there high background in the data?	Be sure to start with viable cells. Check that the cells/nuclei are intact by an automated cell counting method such as the Countess II or by Trypan Blue staining and a hemocytometer.
Will the negative control produce a library?	Yes, the negative control IgG will produce a library.

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