# Automated CUT&RUN brings scalable epigenomic profiling to precision medicine

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## Understanding chromatin dynamics is central to advancing precision medicine, but epigenetics is challenging to study

- Precision medicine has largely focused on Genome Wide Association Studies (GWAS) and transcription; however, variants outside of protein coding regions of the genome (>90%) and environmental factors drive disease pathogenesis
- **Epigenomics is the solution**: Mapping the location of histone post-translational modifications (PTMs) and chromatin-associated proteins, such as transcription factors, provides **molecular insights that are central to cell fate and function**
- However, existing epigenomic technologies, such as **ChIP-seq**, are limited by high costs, poor sensitivity & reliability, and complicated sample prep
- These challenges have precluded epigenomic analysis for personalized medicine

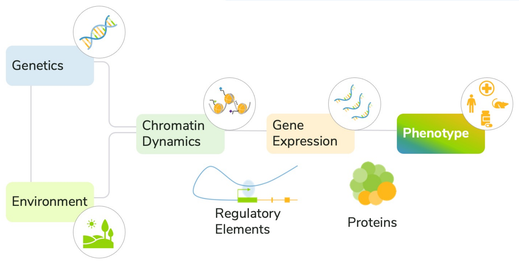



**Figure 1.** The convergence of genetics, environment, transcription, and chromatin structure on the development of disease phenotypes is complex. Current approaches generally fail to account for these diverse factors, making it difficult to predict disease progression and response to treatment.

## CUTANA™ CUT&RUN assays provide important advantages compared to ChIP-seq

### (A) CUT&RUN Workflow




### (B) CUT&RUN generates higher quality data using a fraction of the cells and sequencing costs vs. ChIP-seq

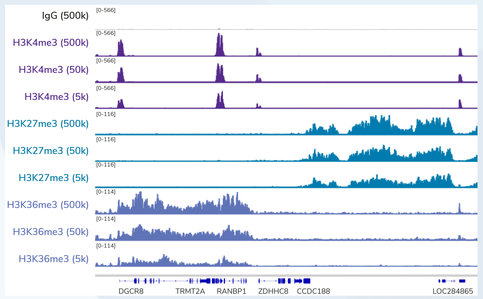
| Comparison | ChIP-seq | CUTANA™ CUT&RUN |
|---|---|---|
| Required cells | > 1 million | 5,000 - 500,000 |
| Input | Fragmented chromatin | Intact cells or nuclei |
| Compatible targets | Histone PTMs & chromatin-associated proteins | Histone PTMs & chromatin-associated proteins, including difficult ChIP targets |
| Sequencing depth | > 30 million reads | 3-8 million reads |
| Experimental throughput | Low | High |
| Signal-to-Noise | Low | High |
| Assay Automation | Difficult | Yes |
| Cost per Reaction | $$$ | $ |

**Figure 2.** Overview of the CUTANA CUT&RUN workflow and advantages compared to ChIP-seq. Because CUT&RUN releases antibody-bound fragments into solution (**A**), it has improved signal-to-noise even with significantly reduced cell numbers and sequencing depth (**B**).

## Automated CUTANA™ CUT&RUN assays enable ultra-sensitive epigenomics from low cell numbers

- Reduced variance and reaction volumes with 96-well liquid handling robots
- Standardized workflow for native and cross-linked cells and nuclei

### (A) High signal-to-noise down to 5k cells




### (B) Profiles maintained across inputs

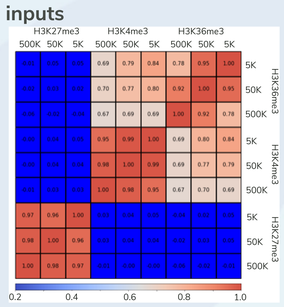



**Figure 3.** Automated CUTANA CUT&RUN (autoCUT&RUN) generates comparable mapping for various histone PTMs using decreasing amounts of K562 cells (**A**). A Pearson correlation matrix (**B**) shows high concordance across cell numbers for each target.

## SNAP-CUTANA™ Spike-in Controls and highly specific antibodies are key to autoCUT&RUN

### (A) SNAP-CUTANA Spike-ins for CUT&RUN

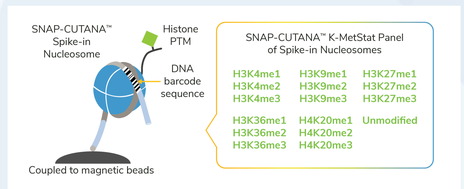



**Figure 4A:** SNAP-CUTANA Spike-ins are panels of DNA-barcoded nucleosomes that are:

- Processed alongside sample as an ideal internal control
- Relative on- and off-target recovery validates antibody specificity and flags failed reactions

### (B) SNAP Spike-ins identify specific histone PTM antibodies for CUT&RUN

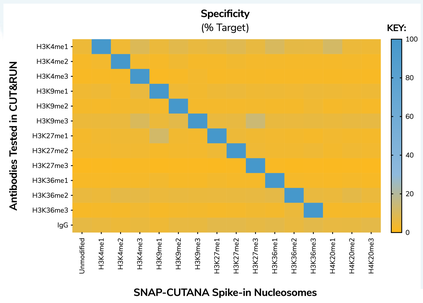
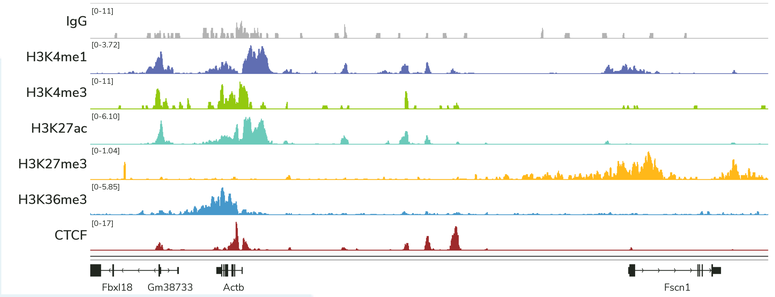



**Figure 4B:** SNAP-CUTANA Spike-ins identify best-in-class CUT&RUN antibodies for histone lysine methylation:

- In the heatmap, each row is a CUT&RUN antibody
- Columns show recovery of spike-ins relative to the on-target PTM (yellow indicates low recovery, blue shows cross-reactivity >20%)

## Epigenomic fingerprinting of immune cell identity using autoCUT&RUN

### (A) Broad target profiling offers a detailed view of cell state




### (B) Scalable epigenomics enables cell-type specific characterization

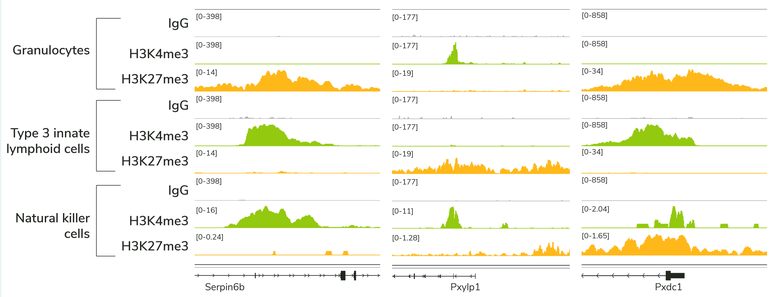



**Figure 5.** High-resolution profiling of FACS-isolated type 3 innate lymphoid cells (ILCs) using autoCUT&RUN identifies unique genomic compartments, including active regulatory elements (H3K4me1, H3K27ac), promoters (H3K4me3), and gene bodies (H3K36me3), as well as repressed genes (H3K27me3) and transcription factor binding sites (CTCF) (**A**). Comparison of target maps for FACS-isolated primary mouse granulocytes, type 3 ILCs, and natural killer cells (Ly49H+) reveals distinct H3K4me3 (promoters) and H3K27me3 (repressed genes) profiles (**B**). All cells prepared and provided by ImmGen, assayed at 10,000 nuclei per reaction.

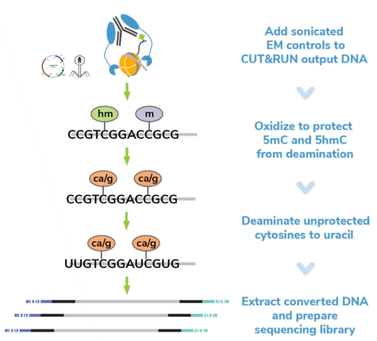
## Conclusions

- **Epigenomics is central to understanding regulation of cell state.** Ultra-sensitive CUTANA CUT&RUN assays offer high-resolution chromatin mapping previously unattainable with historical methods (ChIP-seq).
- EpiCypher's **automated CUT&RUN offers high throughput and cost-effective chromatin mapping**, ideal for low cell numbers and/or scaled clinical projects.
- These efforts are bolstered by **quantitative SNAP-CUTANA Spike-in Controls and highly specific antibodies**, which are crucial for automated epigenomics.
- Analysis of primary immune cells highlights the potential of autoCUT&RUN for deciphering personalized disease etiology at scale.

# Integrating CUT&RUN with DNA methylation analysis enables high-throughput multiomics

## A simple conversion of methylation on CUT&RUN-enriched DNA offers powerful multiomic insights

### (A) CUT&RUN-EM workflow




### (B) CUT&RUN-EM uncovers differential colocalization of PTMs and DNAme

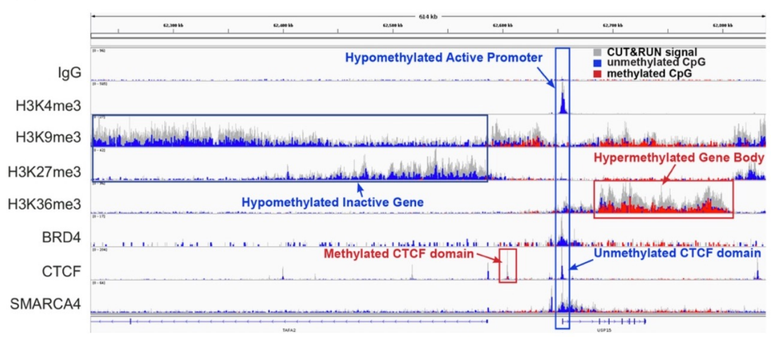



**Figure 6.** CUT&RUN-EM, the combination of CUT&RUN and enzymatic methyl (EM)-seq library conversion, only requires a few additional steps to CUT&RUN / library prep workflows (**A**). This truly multiomic analysis reveals the colocalization of DNA methylation (DNAme) and chromatin targets (histone PTMs or proteins). Genome browser tracks of CUT&RUN-EM reactions profiling various PTM and protein targets reveal differential DNAme levels at various genomic regions (e.g., hypermethylation at gene bodies / hypomethylation at promoters). CUT&RUN signal is in gray, CpGs in blue, and methyl-CpGs in red.

## Reader-targeted CUT&RUN recapitulates whole genome DNAme sequencing

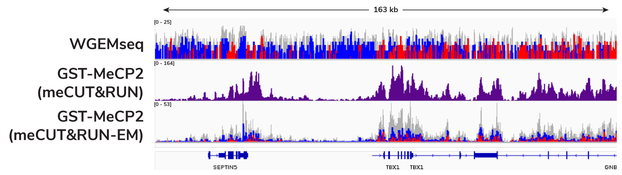



**Figure 7.** Targeted DNA methylation sequencing using the MeCP2 DNAme-binding domain in CUT&RUN (meCUT&RUN) shows similar results to whole-genome enzymatic methyl-seq (WGEMseq), with a 100-fold reduction in sequencing depth. MeCP2 enrichment (purple) localizes to areas of methyl-CpG enrichment over non-methyl-CpG (red vs. blue in WGEMseq). To enable base-pair DNAme resolution, meCUT&RUN is paired with EM-seq (meCUT&RUN-EM), confirming preference of meCP2 for methylated DNA.

## See epigenomics in Precision Medicine

- Overcome drug resistance PMID: 37541244
- Drug action characterization PMID: 37557181
- Reveal risk variant mechanisms PMID: 35649373
- DNAme-driven Rx selection PMID: 32348734

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