Development of a high-throughput CUT&RUN platform for epigenomic mapping of rare primary immune cells

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Epigenetic regulation is central to cell and gene therapy, but has been challenging to study

- Many genomic strategies for cell & gene therapy focus on transcription; however, RNA-seq reveals the **outcomes** – not driving **mechanisms**
- **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 cell & gene therapy

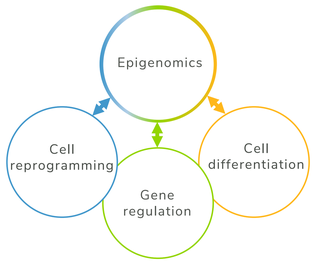
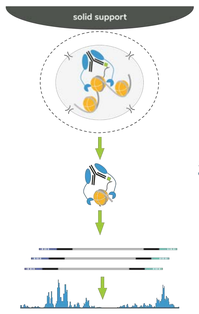


Figure 1: Understanding epigenetic regulation is critical to successful cell and gene therapy applications:

- iPSCs
- CAR T-cells
- T cell exhaustion
- dCas9/Cas9 targeting

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

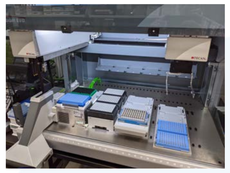
Platform Comparison	ChIP-seq	CUTANA™ CUT&RUN
Required Cells	>1 million	5,000- 500,000
Ideal for Profiling	Histone PTMs, TFs	Histone PTMs, TFs & chromatin remodelers
Sequencing Depth (Reads)	>30 million	3-8 million
Experimental Throughput	Low	High
Signal-to-Noise	Low	High
Assay Automation	Difficult	Yes

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: high-throughput, customized epigenomics

(A) Reduces hands-on time by half while increasing throughput >8x

Freedom EVO100 robotics setup



Platform Comparison	Standard CUT&RUN	Automated CUT&RUN
Length of protocol	4 days	2.5 days
Hands-on time (hours)	17	8.5
Reactions per experiment	24	96
Experiments per week	1x	2x
Total reactions per week	**24**	**192**

(B) Processes up to 384 CUT&RUN reactions per week

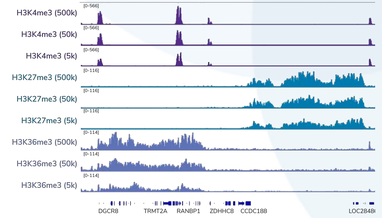
Optimization steps:

- Miniaturized reaction volumes and sequencing library prep for 96-well plates
- Standardized workflow for native & cross-linked cells & nuclei
- Developed spike-in controls to monitor workflows, validate antibodies, and flag failed reactions

Buffer optimization reduces sample loss

autoCUT&RUN enables ultra-sensitive epigenomic profiling from low cell numbers

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



(B) Reliable signal across varying cell numbers

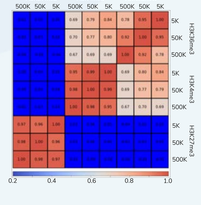


Figure 4. Automated CUTANA CUT&RUN (autoCUT&RUN) was used to generate maps for various histone PTMs using decreasing amounts of K562 cells (**A**). Pearson correlation matrix of H3K4me3 data shows high concordance across cell numbers for each target (**B**).

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

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

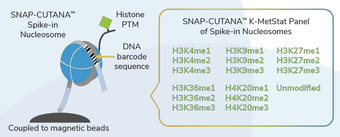


Figure 5A: SNAP-CUTANA Spike-ins are panels of DNA-barcoded nucleosomes that are:

- Added to CUT&RUN just prior to antibody addition
- Processed alongside sample as an ideal internal control
- Recovery of on- and off-target spike-ins determines experimental success

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

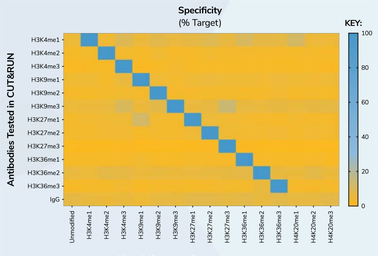


Figure 5B: SNAP-CUTANA Spike-ins were used to identify best-in-class CUT&RUN antibodies for widely studied histone lysine methylation marks:

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

Unique validation strategy enables reliable profiling of transcription factors and chromatin-associated proteins

Validation of EpiCypher antibody to FOXA1

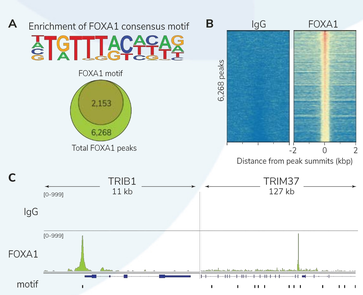


Figure 6: Our stringent validation protocol involves multiple genome-wide analyses to ensure:

- Peak and motif enrichment consistent with biological function (**A, C**)
- High signal over background in genome-wide analyses (**B**)
- Reliable performance across production lots
- FOXA1 antibody (EpiCypher 13-2001) data are shown

High quality antibodies enable low-input autoCUT&RUN experiments

Robust profiling for multiple targets down to 10,000 nuclei

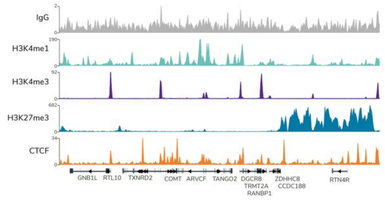


Figure 7: Genomic tracks were generated by autoCUT&RUN using 10,000 lightly cross-linked K562 nuclei and EpiCypher-validated antibodies to H3K4me1 (active regulatory elements), H3K4me3 (active promoters), H3K27me3 (repressed genes), and CTCF (widely studied transcription factor). See epicypher.com/antibodies-complete-list/ for antibodies.

Proof-of-concept: Robust profiling using 10,000 mouse immune cells

Optimized for lightly cross-linked nuclei from mouse B cell line

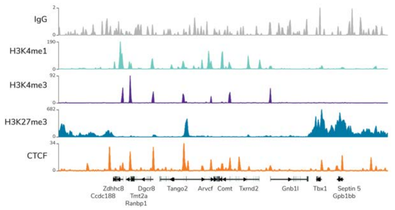


Figure 8: autoCUT&RUN is reliable for immune cell profiling. autoCUT&RUN was performed using 10,000 lightly cross-linked mouse B cell nuclei (provided by the ImmGen Consortium) and same antibodies as in **Figure 7**.

Conclusions

- **Epigenomics is central to understanding gene regulatory processes**, but historical methods (ChIP-seq) are unreliable
- Ultra-sensitive CUTANA CUT&RUN assays are poised to dramatically change the field, improving access to high-resolution chromatin mapping
- EpiCypher developed **automated CUT&RUN for high throughput and cost-effective chromatin mapping**, ideal for studying cell & gene therapy at scale
- These efforts are bolstered by **quantitative SNAP-CUTANA Spike-in Controls and highly specific antibodies**, both of which were crucial to optimizing autoCUT&RUN for immune cell profiling

autoCUT&RUN defines immune cell differentiation pathways for advanced cell & gene therapy research

- Collaboration with ImmGen Consortium
- Each assay only required 10,000 FACS-sorted cells
- Compatible with stimulated and cross-linked cells
- Standardized sample handling improved yields
- Cell-type specific peaks detected – useful for studying cell differentiation

Uncover cell type-specific gene regulatory profiles

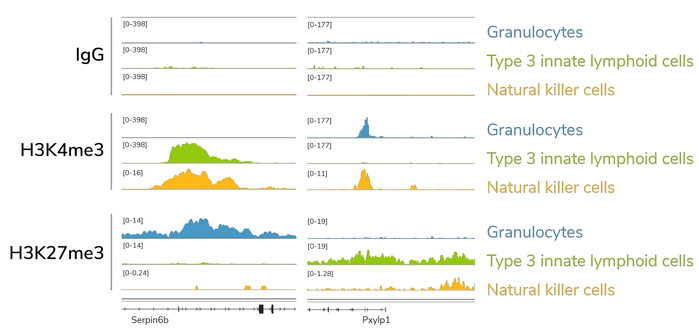


Figure 9. autoCUT&RUN reveals distinct H3K4me3 (marks active promoters) and H3K27me3 (denotes repressed genes) profiles across FACS-sorted primary mouse granulocytes, type 3 innate lymphoid cells, and nature killer cells (Ly49H+), provided by ImmGen. 10,000 nuclei were used per reaction.

In-depth studies of individual immune cell populations reveals novel biology

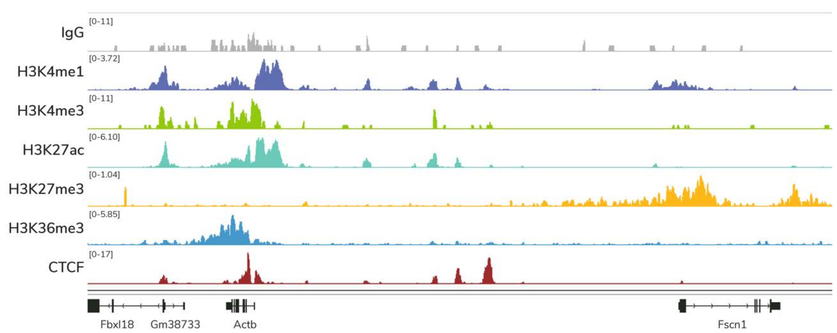


Figure 10. High-resolution profiling of FACS-sorted type 3 ILCs using autoCUT&RUN identifies unique genomic compartments, including active regulatory elements (H3K4me1, H3K27ac), promoters (H3K4me3), and gene bodies (H3K36me3), as well as repressed genes (H3K27me3) and transcription factor binding sites (CTCF). 10,000 nuclei (from ImmGen) were used per reaction.

Applications of CUTANA assays

- Exhausted T cells (PMID: 35930654)
- CAR T cell expansion (PMID: 36944333)
- dCas9/Cas9 targeting (PMID: 35849129)