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

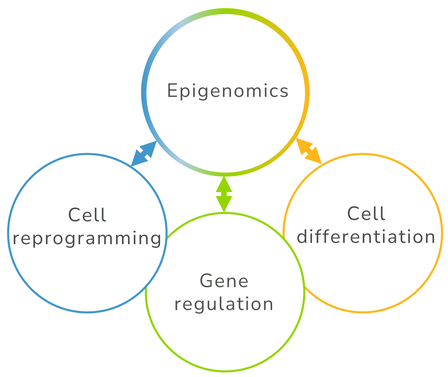


Figure 1: Understanding epigenetic regulation is critical to successful cell and gene therapy applications:

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

CUTANA™ CUT&RUN is a novel workflow that enables streamlined epigenomic mapping

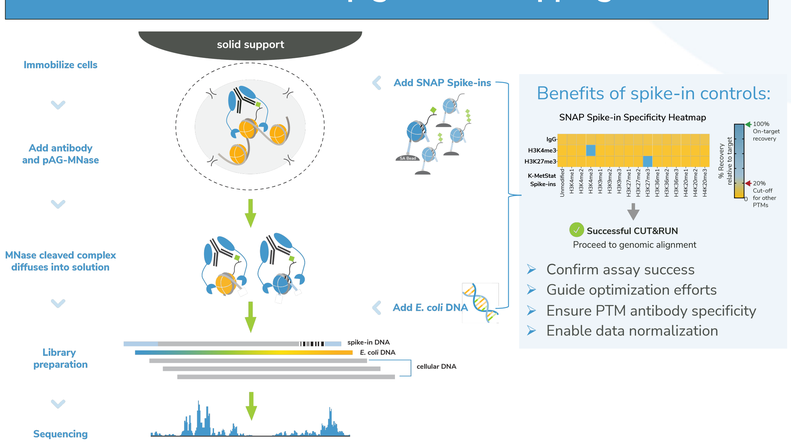
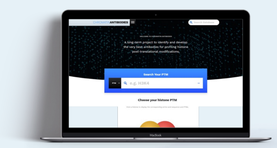


Figure 2. CUT&RUN uses a streamlined workflow to release antibody-bound chromatin into solution, leaving background in bead-immobilized cells. Compared to historical ChIP-seq assays, CUT&RUN generates **higher resolution data with >100-fold reduced cell inputs and >10-fold reduced sequencing depth**. Defined nucleosome controls (SNAP-CUTANA™ Spike-ins) enable assay standardization.

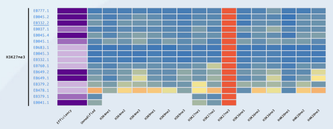
Defined nucleosome controls identify specific and efficient antibodies needed for reliable epigenomics

A. >70% **of histone PTM antibodies are unfit for genomic mapping... but good ones do exist!**

ChromatinAntibodies.com



Search your PTM or Search your antibody

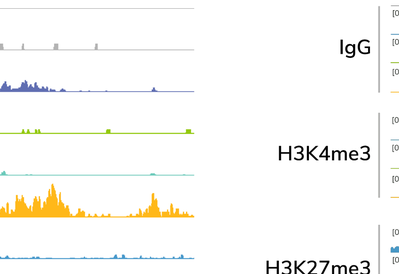


SNAP-ChIP field survey of PTM antibodies:
PASS = <20% cross-reactivity; >5% recovery

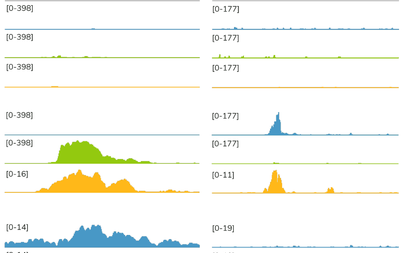
Total	Antibodies tested	392
	Failure rate	**71%**
Most cited antibodies (top 10 studied PTMs)	Antibodies tested	24
	# Citations	4,751
	Failure rate	**79%**

B. SNAP-CUTANA™ spike-ins identify best-in-class CUT&RUN antibodies

High Specificity:
SNAP spike-in panel testing



High Efficiency:
Peak overlay at **standard** & **10x reduced** cells



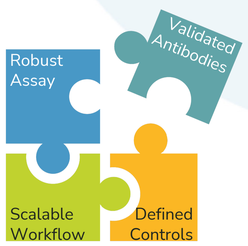
H3K9ac Antibody
X 75% of peaks lost
= **Low efficiency**

H3K4me1 Antibody
✓ 84% of peaks retained
= **High efficiency**

Figure 3. A field survey of histone PTM antibodies shows that the vast majority lack the specificity and efficiency required for reliable genomic mapping (**A**). As the field transitions to CUT&RUN, identifying reliable reagents is key to enable new insights (**B**).

Assembling the pieces for automation: Deploying epigenomics at unprecedented scale & sensitivity

ChIP-seq proved difficult to automate - CUT&RUN empowers epigenomics at scale.



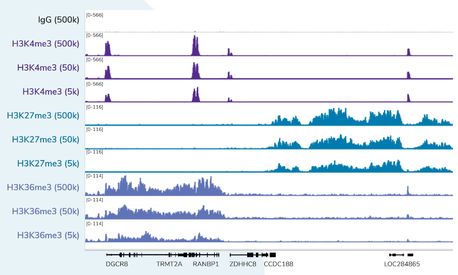
Platform comparison	ChIP-seq	CUTANA™ CUT&RUN	Implications for automation
Sample input	Fragmented chromatin	Intact cells or nuclei	Streamlined workflow
Required cells	>1 million	500k - 5k	Take precious samples further
Defined controls	Uncommon	SNAP Spike-ins	Standardized protocols
Seq depth (reads)	>30 million	3-5 million	Greater multiplexing
Assay cost (per reaction)	~$225	~$72	70% cost savings
Signal-to-noise	Low	High	Better data quality
Experimental throughput	Low	High	Compatible with 96-well plates

autoCUT&RUN enables robust mapping of chromatin-associated proteins at low cell inputs

A. Automation halves hands-on time and increases throughput >8X

- End-to-end optimization for sample prep to library QC
- Reduced variance and reaction volumes with 96-well liquid handling
- Standardized workflow for native and cross-linked cells and nuclei
- Buffer optimization for improved sample handling

B. High signal-to-noise down to 5k cells



C. Reliable signal across inputs

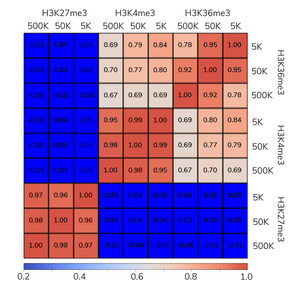
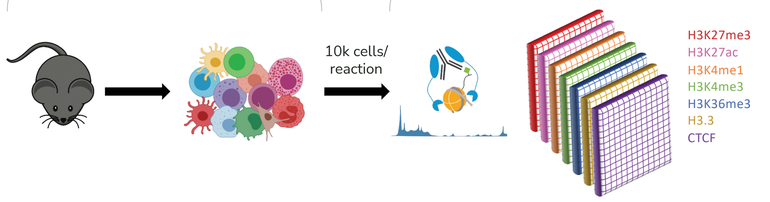


Figure 4. The optimized automated CUTANA™ CUT&RUN (autoCUT&RUN) protocol (**A**) generates comparable maps for various histone PTMs using decreasing amounts of K562 cells (**B**). A Pearson correlation matrix (**C**) shows high concordance across cell numbers for each target.

Application of autoCUT&RUN to generate reference epigenomic maps of the mouse immune system

ImmGen
80-100 Murine Immune Cell Subtypes

EpiCypher
Multi-dimensional feature mapping with CUT&RUN



- Robust, cost-effective autoCUT&RUN assays were used to map **7 distinct chromatin proteins in >100 unique cell types**
- Specific and efficient SNAP-Certified™ Antibodies enabled profiling from **10k FACS-sorted mouse primary immune cells** per reaction
- SNAP Spike-in Controls were used to **monitor sample integrity and confirm reaction success** in 96-well plates
- Generated **>2,200 CUT&RUN profiles** in this multi-site collaboration

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

Broad target profiling provides a detailed view of cell state

Figure 5. autoCUT&RUN profiling of FACS-sorted type 3 ILCs (10k cells/reaction) identifies unique chromatin features, including **poised/active enhancers** (H3K4me1/H3K27ac), **active promoters** (H3K4me3), **gene bodies** (H3K36me3), **repressed genes** (H3K27me3), and **transcription factor binding** (CTCF).

Scalable epigenomics enables cell type characterization

Figure 6. autoCUT&RUN reveals distinct H3K4me3 (**active promoters**) and H3K27me3 (**repressed genes**) profiles across FACS-sorted primary mouse granulocytes, type 3 ILCs, and NK cells (Ly49H+), provided by ImmGen consortium. 10k cells were used per autoCUT&RUN reaction.

CUTANA™ assays in cell & gene therapy research:

- **iPSC profiling** (PMID: 34352411)
- **T-cell exhaustion** (PMID: 35930654)
- **CAR T-cell expansion** (PMID: 36944333)
- **dCas9/Cas9 targeting** (PMID: 35849129)