Epigenomic fingerprinting of limited primary cells using automated CUT&RUN

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Chromatin regulation is central to determining cell fate, but has been challenging to study

- Many genomic strategies for cellular profiling focus on transcription; however, RNA-seq reveals the **outcomes** – not driving **mechanisms**
- **Epigenomics is the solution**: Mapping the location of chromatin features provides mechanistic insights that are central to cell fate and function
- Existing epigenomic technologies lack the necessary controls and scale to enable rigorous investigation of chromatin landscapes in precious samples
- **Improved technologies are needed** to achieve a high-resolution view of chromatin and uncover novel gene regulatory mechanisms (**Figure 1**)

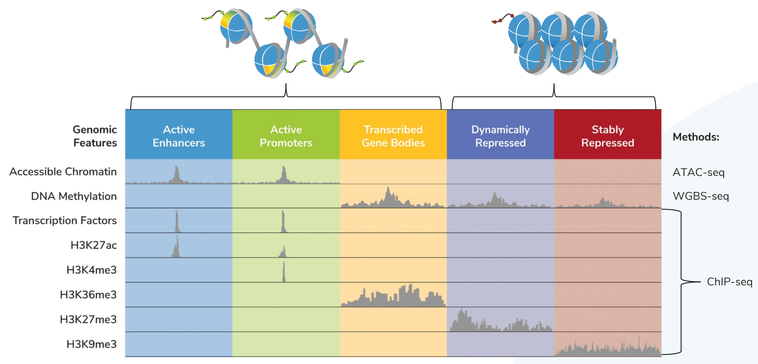


Figure 1. Mapping chromatin-associated proteins and histone PTMs can reveal important regulatory mechanisms governing gene expression, beyond the simplistic open/closed information provided by DNA accessibility and DNA methylation profiling. However, leading technologies (ChIP-seq) have major limitations that impede progress.

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

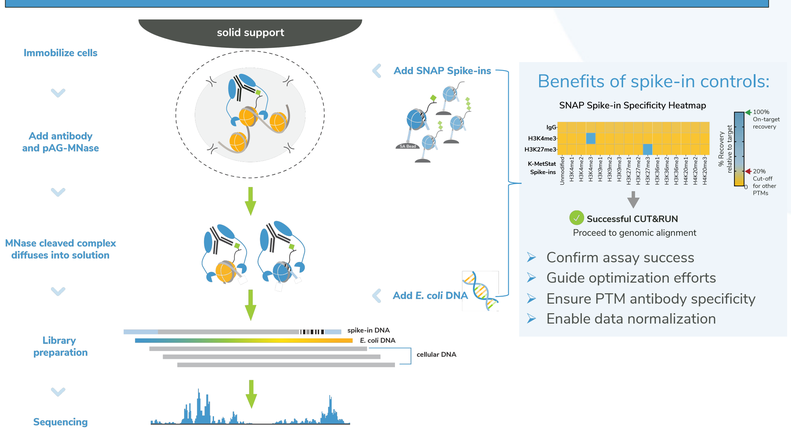
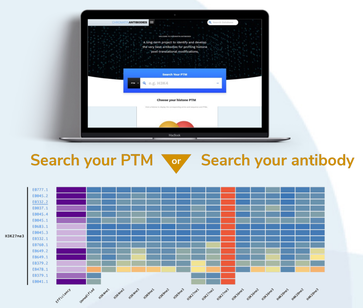


Figure 2. CUT&RUN uses a streamlined workflow to release antibody-bound chromatin into solution, leaving background in bead-immobilized cells. Compared to historical ChIP-seq assays, CUT&RUN generates **higher resolution data with >100-fold reduced cell inputs and >10-fold reduced sequencing depth**. 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

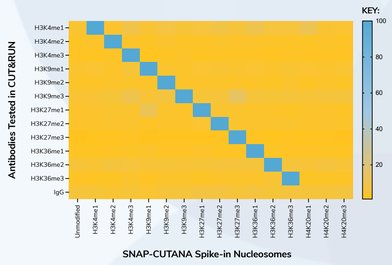


SNAP-ChIP field survey of PTM antibodies:
PASS = <20% cross-reactivity; >5% recovery

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

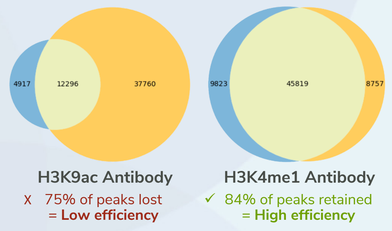


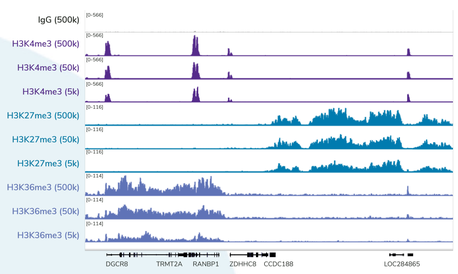
Figure 3. A field survey of histone PTM antibodies shows that the vast majority lack the specificity and efficiency required for reliable genomic mapping (**A**). As the field transitions to CUT&RUN, identifying reliable reagents is key to enable new insights (**B**).

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**

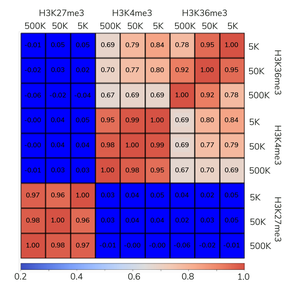
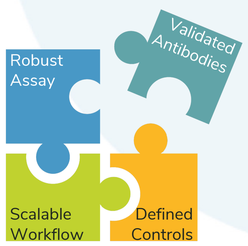


Figure 4. The optimized automated CUTANA™ CUT&RUN (autoCUT&RUN) protocol (**A**) generates comparable maps for various histone PTMs using decreasing amounts of K562 cells (**B**). A Pearson correlation matrix (**C**) shows high concordance across cell numbers for each target.

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

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

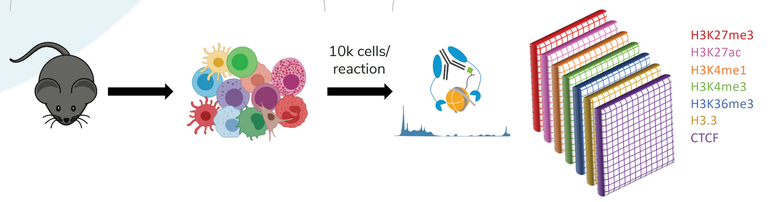


Platform comparison	ChIP-seq	CUTANA™ CUT&RUN	Implications for automation
Sample input	Fragmented chromatin	Intact cells or nuclei	Streamlined workflow
Required cells	>1 million	500k - 5k	Take precious samples further
Defined controls	Uncommon	SNAP Spike-ins	Standardized protocols
Seq depth (reads)	>30 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

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 with precise epigenomic fingerprints

Broad target profiling provides a detailed view of cell state

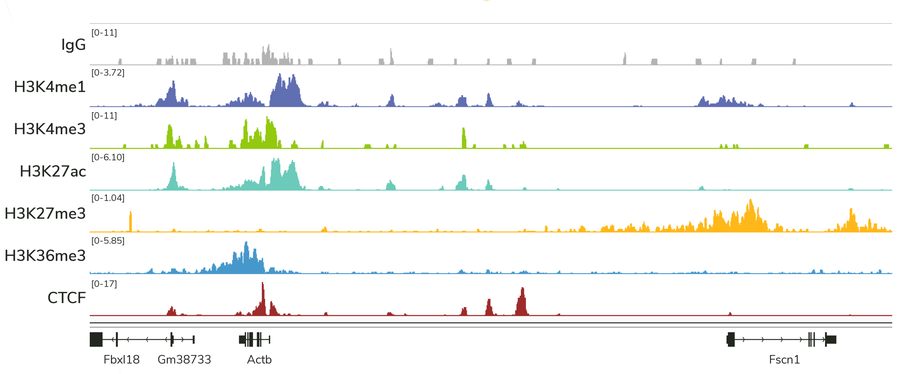


Figure 5. autoCUT&RUN profiling of FACS-sorted type 3 ILCs (10k cells/reaction) identifies unique chromatin features, including **poised/active enhancers** (H3K4me1/H3K27ac), **active promoters** (H3K4me3), **gene bodies** (H3K36me3), **repressed genes** (H3K27me3), and **transcription factor binding**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

Scalable epigenomics enables cell type characterization

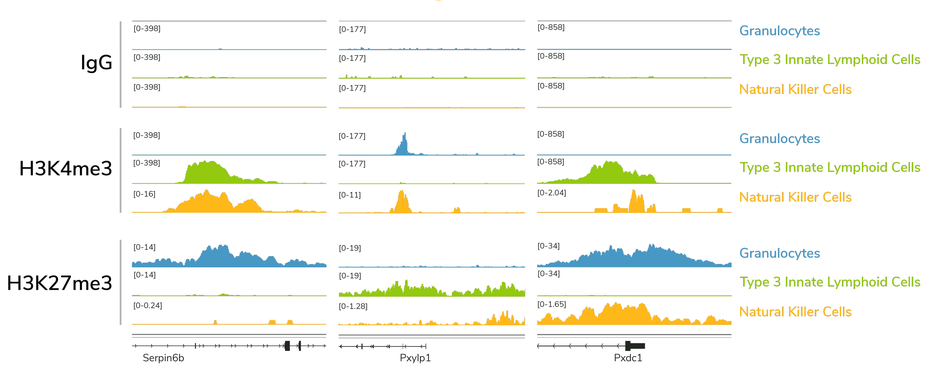


Figure 6. autoCUT&RUN reveals distinct H3K4me3 (**active promoters**) and H3K27me3 (**repressed genes**) profiles across FACS-sorted primary mouse granulocytes, type 3 ILCs, and NK cells (Ly49H+), provided by ImmGen consortium. 10k cells were used per autoCUT&RUN reaction.

See CUTANA™ in action:

- **CUT&Tag in FFPE** (PMID: 37739938)
- **T-cell exhaustion** (PMID: 35930654)
- **SARS-CoV-2 host effects** (PMID: 36918693)
- **CAR T-cell expansion** (PMID: 36944333)
- **dCas-targeted effector** (PMID: 35849129)

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