Development of Quantitative Controls for CUT&RUN Assays

Eva Brill[1], Allison Hickman[1], Bryan J. Venters[1], Andrea L. Johnstone[1], Katherine Novitzky[1], Danielle N. Maryanski[1], Keli L. Rodriguez[1], Ellen Weinzapfel[1], Matthew R. Marunde[1], Carolina P. Lin[1], Ashton Gladney[1], Rachel Watson[1], Zachary B. Gillespie[1], Augustus Adeleke[1], Saarang Gopinath[1], Leslie Lewis[1], Hannah Willis[1], Lu Sun1[1], Vishnu Sunitha Kumary[1], Marcus A. Cheek[1], Matthew J. Meiners[1], James R. Bone[1], Martis W. Cowles[1], Zu-Wen Sun[1], Kami Ahmad[2] & Michael-Christopher Keogh[1]

[1] EpiCypher Inc, [2] Fred Hutchinson Cancer Center

Improved assays and reagents are needed to advance epigenetic research

- Epigenomic mapping for histone post-translational modifications (PTMs) is essential for driving biological discovery
- ChIP-seq is the most widely used epigenomic mapping assay, but has major limitations:
  - X Depends on PTM antibodies – which are notoriously cross-reactive [1,2]
  - X Requires large cell numbers – unsuitable for clinical or rare cell samples
  - X Poor data quality – low signal to noise ratio, poor reproducibility
  - X Expensive and laborious – making it difficult to scale
  - X Lacks defined controls – crucial for reliable, quantitative results
- Defined controls and improved methods are needed to fully leverage epigenomics in innovative research

Nucleosome spike-in controls show that most histone PTM antibodies fail in ChIP-seq

(A) Fully defined ChIP controls mimic physiological targets

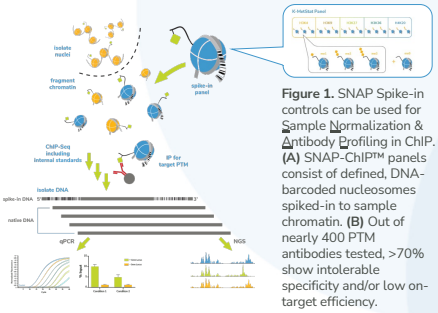


Figure 1. SNAP Spike-in controls can be used for Sample Normalization & Antibody Profiling in ChIP. **(A)** SNAP-ChIP™ panels consist of defined, DNA-barcoded nucleosomes spiked-in to sample chromatin. **(B)** Out of nearly 400 PTM antibodies tested, >70% show intolerable specificity and/or low on-target efficiency.

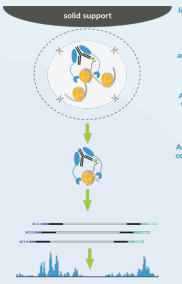
(B) Most commercial antibodies are unfit for epigenomics

Antibodies Tested by SNAP-ChIP™	Lysine Methylation	Lysine Acylation	Total
Total Tested	263	129	392
Failure Rate	74.5%	64.5%	71.2%
$ Spent	$105,200	$51,600	$156,800
$ Wasted	$78,374	$33,282	$111,656
Top Cited: Citations w/"ChIP"	3,720 (N=18)	1,031 (N=6)	4,751 (N=24)
Top Cited: Failure Rate	74.5%	64.5%	79.2%

Data available at: www.ChromatinAntibodies.com[3]

CUTANA™ CUT&RUN has major advantages over ChIP

(A) CUT&RUN Workflow



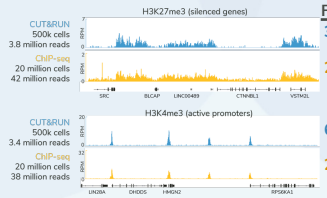
(B) CUT&RUN vs. ChIP-seq

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**).

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

(A) CUT&RUN vs. ChIP-seq: better data, low sequencing costs



(B) CUT&RUN generates reliable profiles down to 5,000 cells

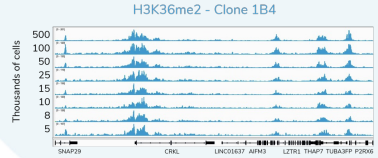


Figure 3. (A) The Fraction of Reads in Peaks (FRiP) score, a measure of signal-to-noise (S:N), is higher in CUT&RUN vs. ChIP-seq, despite using fewer cells and reduced sequencing depths. **(B)** Enrichment profiles are conserved when inputs are titrated from 500k to 5k cells.

SNAP-CUTANA™ Spike-ins are critical controls for reliable chromatin mapping

(A) SNAP Spike-ins for CUT&RUN

(B) SNAP testing identifies specific antibodies for CUT&RUN

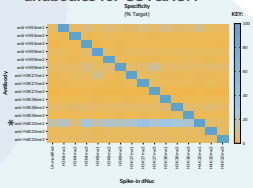
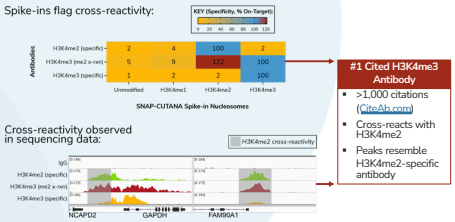


Figure 4. SNAP-CUTANA spike-in controls optimized for use in CUT&RUN (**A**) can be used to identify highly specific antibodies to widely studied histone PTMs (**B**). By spiking them into CUT&RUN workflows just prior to antibody addition, they provide a quantitative readout of on- vs. off-target recovery that predicts non-specific peaks in genomic data (**C**). **Ongoing testing for H4K20me1. Inquire at info@epicypher.com for up-to-date information.*

(C) SNAP Spike-ins predict non-specific recovery in CUT&RUN



Conclusions

- CUTANA™ CUT&RUN is poised to rapidly replace ChIP-seq
- Our data illustrate how CUT&RUN could be applied for epigenomics research, particularly for low sample inputs and/or high-throughput applications
- SNAP Spike-in controls address pervasive antibody specificity problems while enabling a direct readout of assay success and quantitative normalization (*see below!*)

References

1. Shah *et al.* Examining the roles of H3K4 methylation states with systematically characterized antibodies. *Mol. Cell* **72**, 162-177 (2018).
2. Small *et al.* Chromatin Immunoprecipitation (ChIP) to study DNA-Protein Interactions. *Methods Mol. Biol.* **2261**, 323-343 (2021).
3. Maryanski *et al.* Novel nucleosome-based methods for rapid screening and identification of best-in-class antibodies: a community resource to improve genomic mapping. *In preparation.* www.ChromatinAntibodies.com

Ultra-sensitive epigenomics drives biological discovery

Uncover novel biology with highly specific antibodies

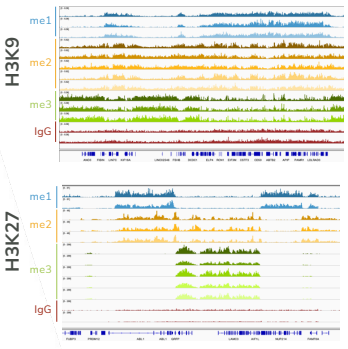


Figure 5. GENOMICALLY DISTINCT = FUNCTIONALLY DISTINCT. The use of highly specific histone PTM antibodies in CUT&RUN (**Figure 4B**) enables novel insights into the histone code. Distinct genomic profiles observed by mono-, di– and tri-methyl states imply distinct biological functions not previously appreciated.

Quantitative readout enables drug & clinical development applications

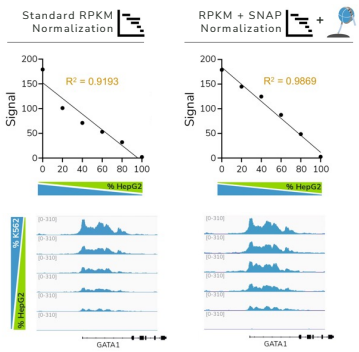


Figure 6. CUT&RUN linearity is improved by SNAP normalization. Cells with distinct lineages were mixed at defined intervals. Linear regression analysis of an H3K4me3 peak shows that SNAP normalization (**right**) shows improved linearity of a K562 cell-specific peak over standard RPKM normalization (**left**).

● **K562 cells** (mesodermal) ● **HepG2 cells** (ectodermal)

SCAN TO DOWNLOAD THIS POSTER NOW