CUTANA™ CUT&Tag : Powerful Platform for Streamlined, Ultra-Sensitive Epigenomics

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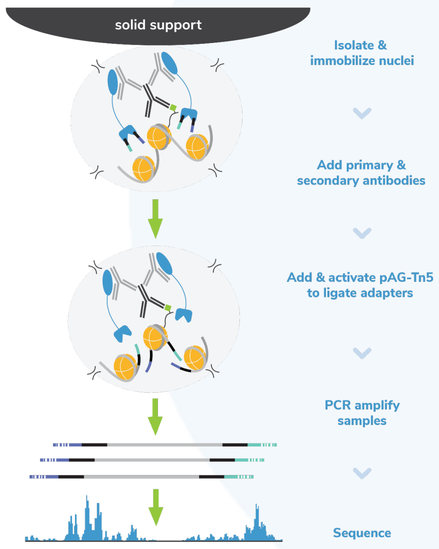
Improved assays and reagents are needed to advance epigenetic research

- 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 Lacks defined controls – crucial for reliable, quantitative results
- Compared to ChIP-seq, CUT&Tag[3] provides higher quality sequencing data with improved sensitivity and dramatically reduced background.

Advantages of CUTANA™ CUT&Tag over ChIP-seq

(A) CUT&Tag Workflow



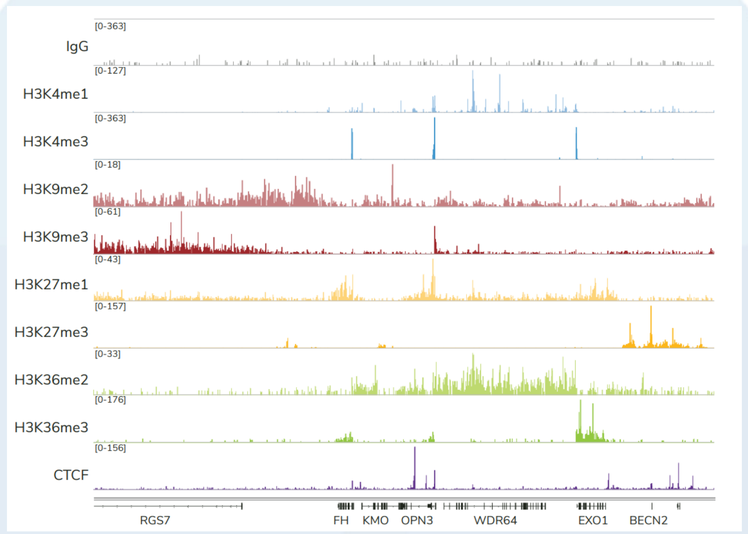
(B) CUTANA™ vs. ChIP-seq

Platform Comparison	ChIP-seq	CUTANA™ CUT&RUN	CUTANA™ CUT&Tag
Required Cells	>1 million	5,000-500,000	10,000-100,000
Ideal for Profiling	Histone PTMs, TFs	Histone PTMs, TFs & remodelers	Histone PTMs
Sequencing Depth (Reads)	>30 million	3-8 million	3-8 million
Experimental Throughput	Low	High	High
Signal-to-Noise	Low	High	High
Library Prep	Standard	Standard	Streamlined Direct-to-PCR

Figure 1. Overview of the CUTANA™ CUT&Tag workflow and advantages compared to ChIP-seq. Because CUTANA™ technologies release antibody bound fragments into solution (**A**), it has improved signal-to-noise even with significantly reduced cell numbers and sequencing depth (**B**). In CUT&Tag, pAG-Tn5 inserts sequencing adapters at antibody bound chromatin in intact nuclei, streamlining library preparation.

CUTANA™ CUT&Tag is ideal for mapping histone PTMs

(A) CUT&Tag delivers robust data across diverse PTM targets



(B) TSS Enrichment

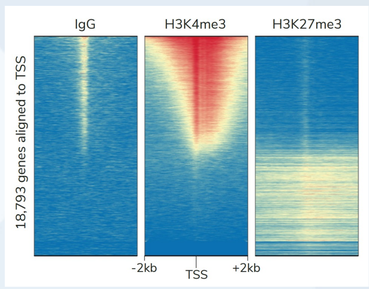


Figure 2. (A) CUTANA CUT&Tag generates high-quality profiles for targets in both active and repressed chromatin regions and select proteins. Rabbit IgG is shown as a negative control. **(B)** Expected results from CUTANA CUT&Tag assays. Genes are aligned across targets and ranked by H3K4me3 intensity at transcription start sites (TSSs) from top (high signal, red) to bottom (low signal, yellow).

CUT&Tag excels at epigenetic profiling from low cell numbers

(A) CUT&Tag generates reliable profiles down to 10K cells

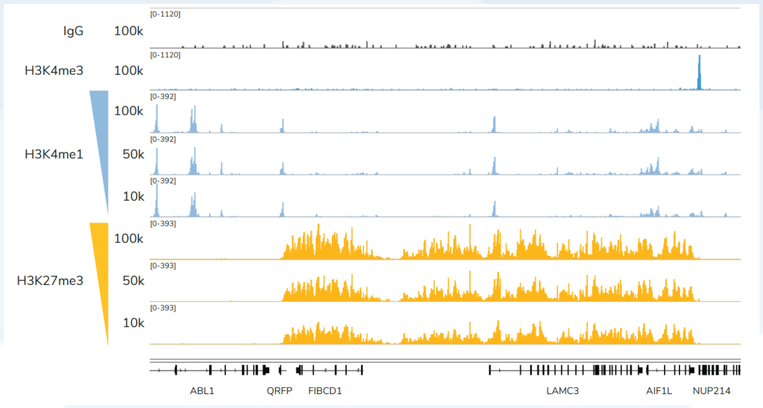
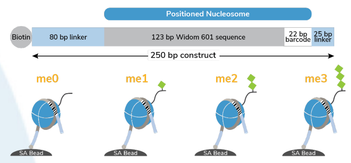


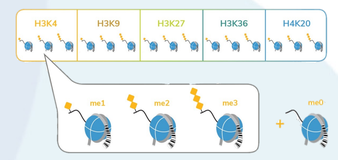
Figure 3. (A) CUTANA CUT&Tag enables reliable chromatin profiling from low cell numbers. CUT&Tag was used to map H3K4me1 and using decreasing numbers of K562 cells. Data quality at 10,000 cells is comparable to standard inputs of 100,000 cells.

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

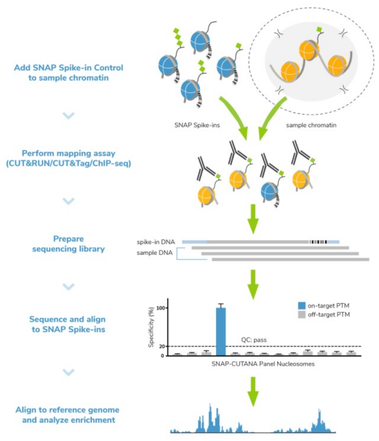
(A) SNAP Spike-ins for CUTANA Technologies



(B) SNAP testing identifies specific antibodies



(C) Spike-In Workflow



(D) SNAP-CUTANA Specificity Heatmap

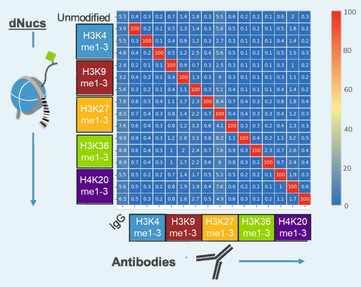


Figure 4. SNAP CUTANA controls can be used for Sample Normalization & Antibody Profiling in CUTANA assays. **(A)** SNAP-CUTANA™ panels consist of defined, DNA-barcoded nucleosomes spiked-in to sample chromatin. **(B)** KMetStat panel that would be processed alongside sample as an ideal internal control. **(C)** Panel is spiked into CUT&RUN workflows just prior to antibody addition. **(D)** They provide a quantitative readout of on- vs. off-target recovery that predicts non-specific peaks in genomic data.

Conclusions

- CUTANA™ technologies (CUT&RUN and CUT&Tag) are poised to rapidly replace ChIP-seq.
- CUT&Tag uses a streamlined protocol to produce high quality data for diverse targets with low cell number requirements and reduced sequencing costs.
- SNAP Spike-in controls inform antibody specificity and monitor assay success.
- CUTAC recapitulates published ATAC-seq datasets but with significantly improved sensitivity and signal-to-noise.

References

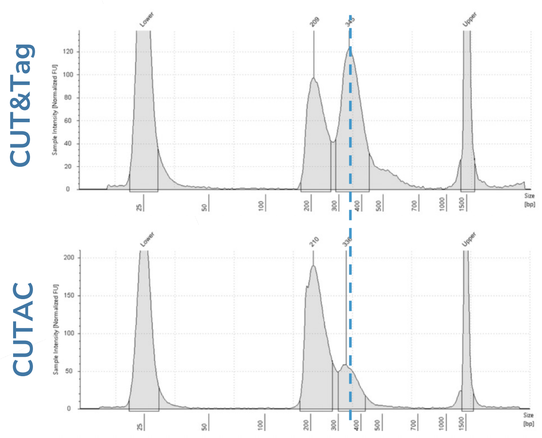
1. Shah *et al*. Examining the roles of H3K4 methylation states with systematically characterized antibodies. *Mol. Cell* **72**, 162-177 (2018).
2. Small *et al*. Chromatin Immunoprecipitation (ChIP) to study DNA-Protein Interactions. *Methods Mol. Biol.* **2261**, 323-343 (2021).
3. Kaya-Okur HS et al. CUT&Tag for efficient epigenomic profiling of small samples and single cells. *Nat Commun* 10, 1930 (2019).
4. Steven HenikoffJorja G HenikoffHatice S Kaya-OkurKami Ahmad (2020) Efficient chromatin accessibility mapping in situ by nucleosome-tethered tagmentation. *eLife* 9:e63274.

CUTAC : Identifying Open Chromatin with Modified CUT&Tag

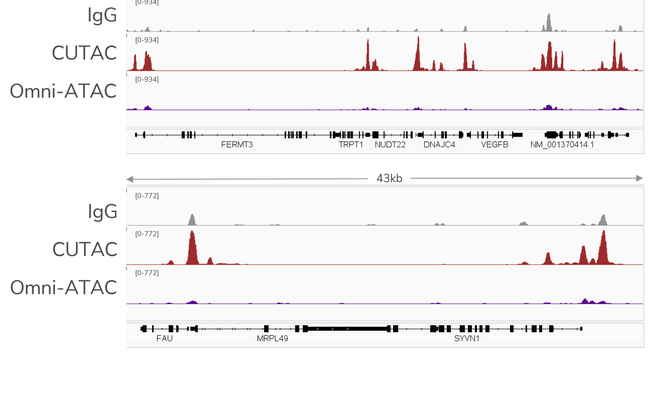
(A) CUTAC conditions differ from CUT&Tag

Platform Comparison	CUT&Tag	CUTAC
Feature Enrichment	Histone PTMs	Chromatin Accessibility
Detection Reagent	PTM-specific Antibody	H3K4me2 Antibody
Tagmentation Salt Conditions	300mM NaCl, 10mM $MgCl_2$	0mM NaCl, 5mM $MgCl_2$
Tagmentation Time	1hr	20min
Tagmentation Temperature	37C	37C

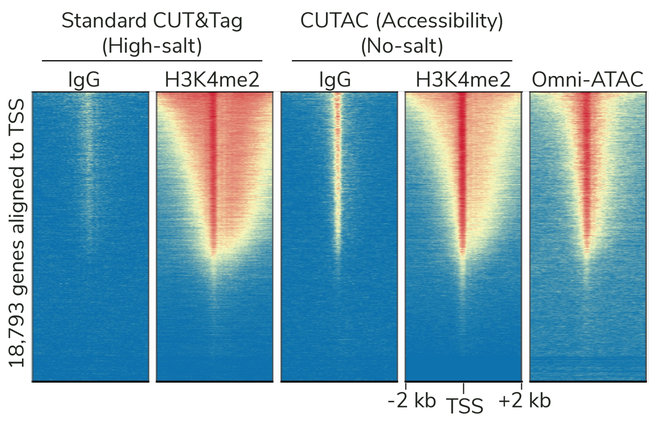
(B) CUTAC releases smaller genomic fragments than CUT&Tag



(C) CUTAC shows stronger signal than Omni-ATAC



(D) CUTAC, CUT&Tag, and Omni-ATAC enrichment relative to TSS



(E) Peak calling comparisons across CUTAC, CUT&Tag, and Omni-ATAC

Assay	FRiP (%)
H3K4me2 CUT&Tag	63.5%
CUTAC	64.1%
Omni-ATAC	11.3%

FRiP = Fraction of reads in peaks, measure of signal-to-noise

Figure 5. CUTAC[4] is an exciting new application of CUT&Tag that identifies open chromatin regions by restricting tagmentation to accessible DNA at nearby transcriptionally active chromatin. **(A)** CUTAC experiments use less salt and a shorter tagmentation time compared to CUT&Tag. **(B)** TapeStation traces from CUTANA CUT&Tag and CUTAC libraries prepared using H3K4me2 antibodies. While CUT&Tag libraries predominantly enrich for mononucleosome-sized fragments (~300bp fragments), CUTAC libraries enrich for smaller fragments (~200bp). **(C)** IGV screenshots of CUTAC and Omni-ATAC data compared to non-specific IgG. **(D)** Heatmaps show signal relative to TSS in each assay. Gene rows are aligned across conditions. **(E)** Venn diagram showing peak overlap between H3K4me2 CUT&Tag, CUTAC, and Omni-ATAC-seq. FRiP scores show that CUT&Tag and CUTAC data have higher signal-to-noise vs. Omni-ATAC-seq.

Other Applications of CUT&Tag

SWI/SNF in SARS-CoV-2 Infection
(PMID: 36894709)

CD4+ T cell calibration
(PMID: 37188942)

3D genome in AML
(PMID: 36289338)

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