Profiling histone modifications in single cells to gain insight into the effects of epigenetic drug treatment on tumor cells

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ABSTRACT

Epigenomic remodeling, such as changes in chromatin state, play a crucial role in cancer biology by regulating gene expression programs that drive tumor progression, immune evasion, and therapeutic resistance. Given the heterogeneity commonly present in tumors, single-cell methods are key to understanding epigenetic changes. However, to date, commercialized single-cell assays for assessing chromatin state have been limited to scATAC-seq (single-cell assay for transposase-accessible chromatin using sequencing), which only allows for profiling of open chromatin and is unable to provide more detailed information about the nature of the accessible regions or silenced chromatin.

CUT&Tag (cleavage under targets and tagmentation) enables chromatin profiling at significantly higher sensitivity and reduced sequencing costs compared to ChIP-seq (chromatin immunoprecipitation sequencing). In contrast to ATAC-seq, CUT&Tag can provide more specific information on chromatin states. Similar to ChIP-seq, CUT&Tag targets histone modifications with specific antibodies. Unlike ChIP-seq, DNA is tagmented with a protein A/G-Tn5 fusion enzyme, which inserts sequencing adapters in situ, enabling single-cell resolution. Kaya-Okur et al. showed proof of concept of single-cell CUT&Tag (scCUT&Tag); however, to date, there are no commercialized methods for high-throughput scCUT&Tag.

Here, Takara Bio's Shasta® Single Cell System was used to profile several thousand single cells using scCUT&Tag. With this system, we revealed global cell-type-specific acetylation in A549 cells in response to epigenetic therapy. A549 WT and A549 p53 KO cells were treated with a combination of decitabine (DNMT inhibitor) and panobinostat (pan-HDAC inhibitor) or DMSO (control). scCUT&Tag targeting H3K27ac was performed using the Shasta system. Data was normalized using the SNAP-CUTANA spike-in panel from EpiCypher.

We found that epigenetic therapy treatment increased acetylation of A549 expressed genes compared to DMSO control treatment in both A549 WT and A549 p53 KO cells. Comparison of CUT&Tag data to single-cell gene expression analysis using Shasta Total RNA-Seq corroborated findings in the RNA-seq data, as we noted increased acetylation at promotors and enhancers of genes with increased gene expression.

In summary, Shasta CUT&Tag provides a high-throughput, automated approach to identify genes regulated in response to epigenetic drug treatment of tumor cells at the single-cell level. Integrating single-cell expression data provides deeper insight into cellular regulation, particularly in the context of drug responses in tumor cells.

1 Shasta CUT&Tag workflow

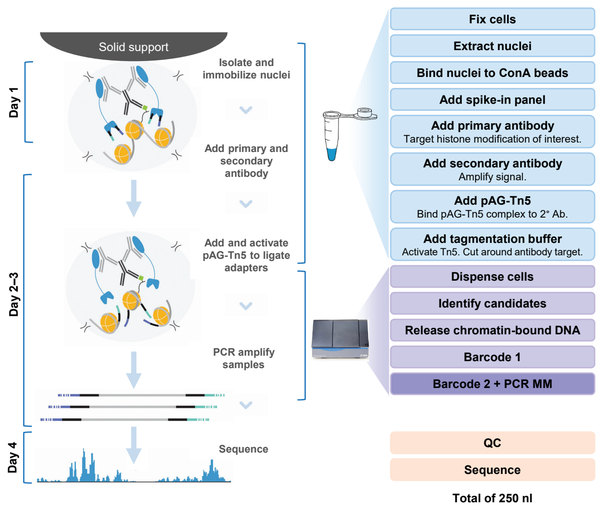
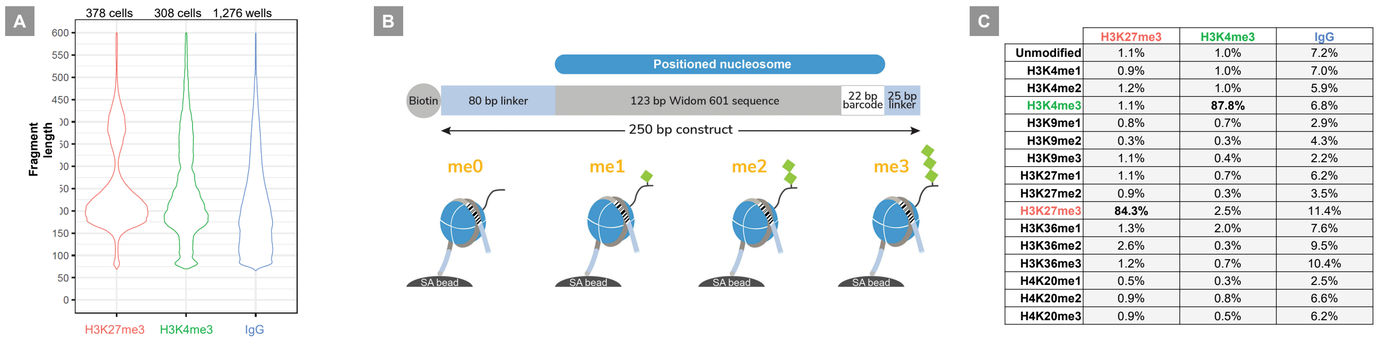


Figure 1. Shasta CUT&Tag workflow. Cells first undergo light fixation followed by nuclear extraction. Nuclei are bound to magnetic beads coated with Conavelin A (ConA). SNAP-CUTANA K-MetStat Panel is added, followed by the primary target antibody. After overnight incubation, a secondary antibody and pAG-Tn5 are added consecutively. Tn5 is activated for in situ tagmentation of chromatin at the target post-translational modification. Until this point, the workflow can be completed in a single tube. Nuclei are stained and dispensed into a 5,184-nanowell chip. Single-cell candidates are identified by imaging. DNA release reagent is added to the chip, which is incubated at 58°C for 1 hr. Next, two indexing primers and the PCR master mix are dispensed into the chip to amplify the libraries while simultaneously incorporating a unique barcode for each single nucleus. The pooled barcoded libraries are ready for Illumina® sequencing after off-chip purification. Light blue = bulk single-tube prep; light purple = 50 nl dispense; dark purple = 100 nl dispense; orange = pooled sample. Image adapted from https://www.epicypher.com/content/images/products/methods/cut-and-tag-methods.png with permission from EpiCypher, Inc.

2 QC metrics: assessment of fragment length and antibody specificity



	H3K27me3	H3K4me3	IgG
Unmodified	1.1%	1.0%	7.2%
H3K4me1	0.9%	1.0%	7.0%
H3K4me2	1.2%	1.0%	5.9%
H3K4me3	1.1%	**87.8%**	6.8%
H3K9me1	0.8%	0.7%	2.9%
H3K9me2	0.3%	0.3%	4.3%
H3K9me3	1.1%	0.4%	2.2%
H3K27me1	1.1%	0.7%	6.2%
H3K27me2	0.9%	0.3%	3.5%
H3K27me3	**84.3%**	2.5%	11.4%
H3K36me1	1.3%	2.0%	7.6%
H3K36me2	2.6%	0.3%	9.5%
H3K36me3	1.2%	0.7%	10.4%
H4K20me1	0.5%	0.3%	2.5%
H4K20me2	0.9%	0.8%	6.6%
H4K20me3	0.9%	0.5%	6.2%

Figure 2. Fragment-length analysis of SNAP-CUTANA K-MetStat Panel highlights excellent performance of antibodies. Panel A. Fragment-length analysis of H3K27me3 and H3K4me3 shows the typical nucleosomal pattern of pseudobulk CUT&Tag reads. Each datapoint represents ~300 single K562 cells. IgG has been processed off the Shasta chip in bulk by releasing the chromatin-bound DNA prior to dispensing on the Shasta system (1,200 sample wells equating to ~1,200 single cells). **Panel B.** Schematic view of the K-MetStat panel, which controls for 15 widely studied methyl states and an unmodified control (me0), with each of the 16 octamers wrapped with two uniquely barcoded DNA templates. Each 250 bp DNA template contains a 123 bp 601 nucleosome positioning sequence (gray; Lowary & Widom, 1998), a unique 22 bp DNA barcode (white; 32 barcodes total), and a 5′ biotin-TEG. The 5′ and 3′ linkers (blue) are compatible with tagmentation by pAG-Tn5. The designer nucleosomes (blue) are individually preconjugated to paramagnetic beads and pooled for convenient use. Image in Panel B was reused from https://www.epicypher.com/content/images/products/nucleosomes/19-1002_graphic.jpeg with permission from EpiCypher, Inc. **Panel C.** SNAP-CUTANA K-MetStat spike-in panel shows high specificity for target antibodies, H3K4me3, and H3K27me3. IgG was included as control. The same spike-in panel has also been used for data normalization in later figures.

3 Comparison of chromatin landscape between bulk, pseudobulk, and single cells

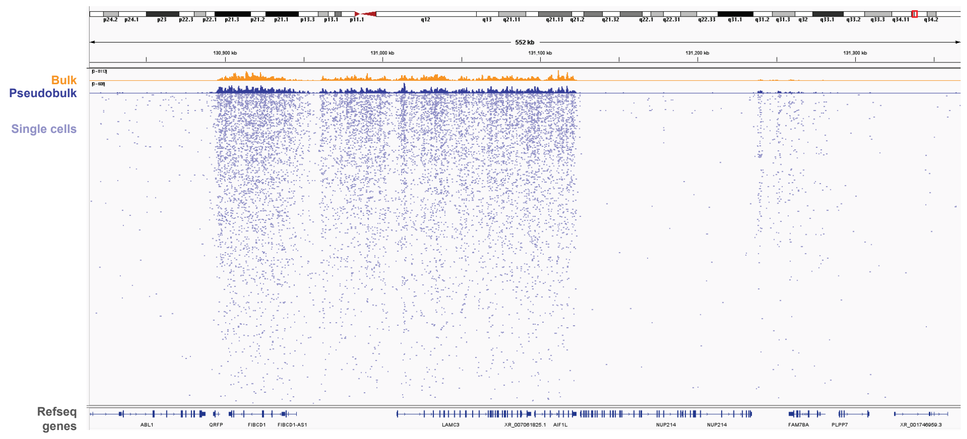


Figure 3. Comparison of chromatin landscape for bulk (100,000 cells), pseudobulk (620 aggregated cells), and 620 single K562 cells for H3K27me3 CUT&Tag. Genome browser tracks show a 552 kb segment of the human genome. Bulk (orange) and pseudobulk (dark blue) tracks are shown as bar charts. Single cells (light blue) are plotted as points for better visibility and ordered by total read counts per cell.

4 Shasta CUT&Tag has a low doublet rate

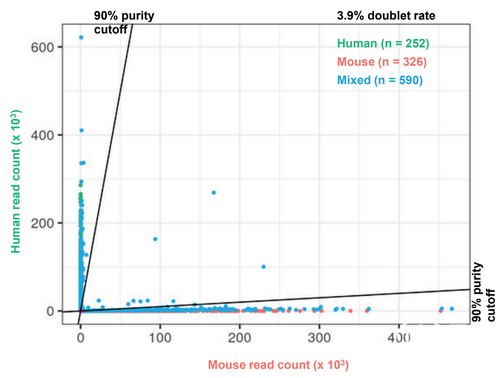


Figure 4. Shasta CUT&Tag has a very low doublet rate as assessed through a barnyard experiment. For this experiment, human (K562) and mouse (3T3) cells were mixed 1:1. Pure human/mouse samples served as controls. Shasta CUT&Tag targeting H3K4me3 was performed for all three samples (human [green], mouse [red], and mixed [blue]) and libraries were sequenced. Barcodes with sequences aligned to either mouse or human reference genomes were considered singlets and assigned to either mouse or human. Barcodes with sequences aligned to both human and mouse reference genomes were considered doublets. Human-mouse doublet rate was 3.9%, with a purity threshold set at 90% and minimum read cutoff of 1,000 read pairs.

5 Shasta CUT&Tag with drug-treated lung cancer cells

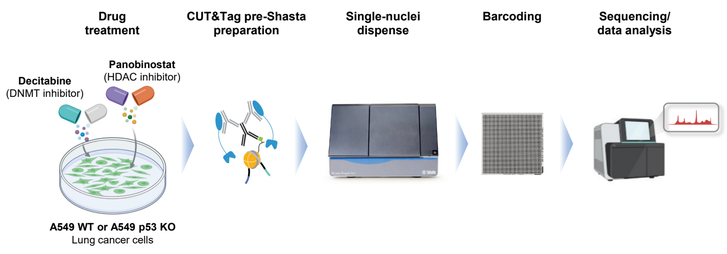


Figure 5. Shasta CUT&Tag workflow on epitherapy-treated lung cancer cells. A549 WT and A549 p53 KO cells were treated for four days with 1 µM decitabine (DNMT inhibitor), followed by two days of combination treatment with 1 µM decitabine and 40 nM panobinostat (pan-HDAC inhibitor). Control samples were treated with 0.05% DMSO for six days. H3K27ac target antibody was used for Shasta CUT&Tag. After tagmentation, cells were stained with DAPI, and each sample was dispensed on a separate 350 nl chip. Chips were imaged to identify single-cell candidates, followed by DNA release, indexing, and PCR amplification of tagmented fragments with unique barcodes. Cells from the two chips were pooled and sequenced on a NextSeq® 500 instrument. Image adapted from https://www.epicypher.com/content/images/products/methods/cut-and-tag-methods.png with permission from EpiCypher, Inc., and created with Biorender.

6 Shasta CUT&Tag reveals epitherapy-induced increase in H3K27ac

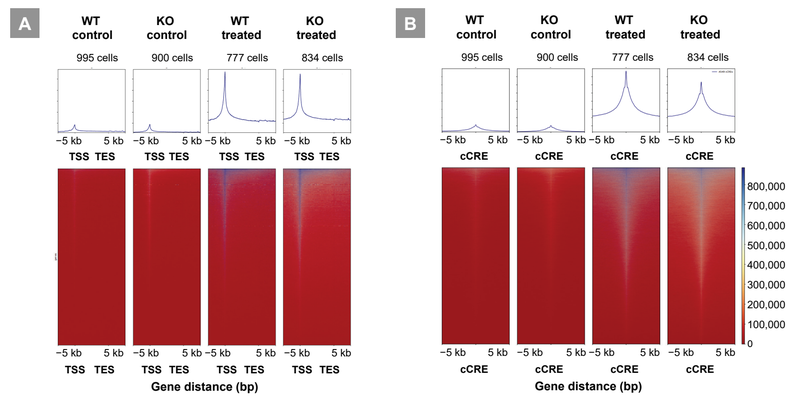


Figure 6. Combination of HDAC inhibitor and DNMT inhibitor increases H3K27ac at transcription start sites (TSSs) and candidate *cis*-regulatory elements (cCREs) compared to controls. Panel A. Pseudobulked H3K27ac read signal after spike-in normalization is shown at genes from GENCODE v47 for the four A549 conditions. Flanking 5 kb regions upstream of the TSS and downstream of the transcription end site (TES) are included. **Panel B.** Pseudobulked H3K27ac read signal after spike-in normalization is shown at cCREs from ENCODE4. Flanking 5 kb regions around cCREs are included to provide local background levels.

7 Correlation of Shasta CUT&Tag with Shasta Total RNA

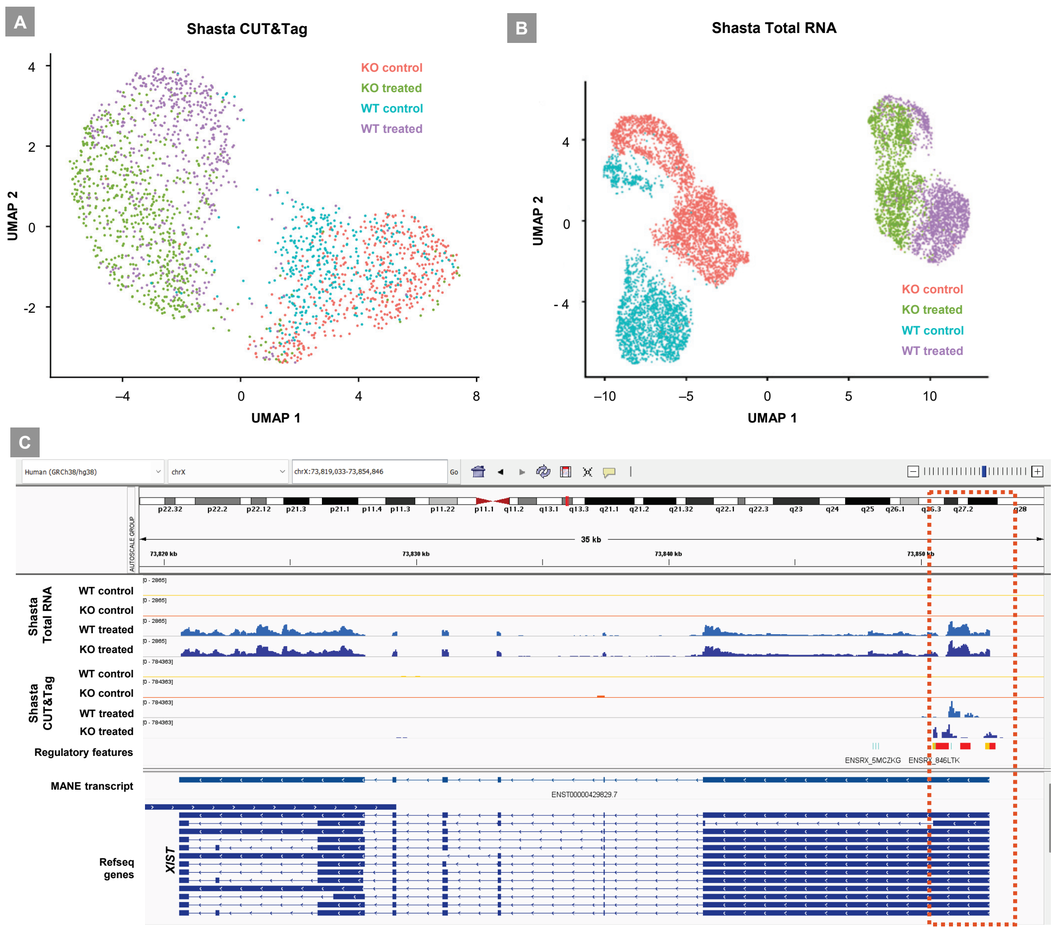


Figure 7. High throughput single-cell gene expression analysis by Shasta Total RNA corroborates Shasta CUT&Tag findings in drug-treated A549 cells. Panel A. UMAP visualization of A549 cells assessed for H3K27ac by Shasta CUT&Tag shows two distinct clusters separating the epitherapy-treated cells from the nontreated cells (2,133 total cells). QC filter: minimum features per cell ≥200; minimum reads per feature ≥10; maximum mitochondrial percent ≤50%. **Panel B.** UMAP visualization of gene expression in 8,561 single cells processed by Shasta Total RNA distinguishes between WT and p53 KO cells. Yet, cells mainly separate by treatment status (epitherapy treated vs. untreated), corroborating the findings of Shasta CUT&Tag. **Panel C.** Genome browser tracks (IGV) for Shasta Total RNA and Shasta CUT&Tag show increased *XIST* gene expression and increased H3K27ac at *XIST*'s promotor/enhancer, respectively, in treated cells. Shasta CUT&Tag samples were normalized with the SNAP-CUTANA K-MetStat spike-in panel and group-autoscaled in IGV. Yellow = A549 WT control; orange = A549 p53 KO control; light blue = A549 WT treated; dark blue = A549 p53 KO treated. Ensemble regulator feature track color codes: red = promoter; yellow = enhancer; light blue = CTCF binding site.

REFERENCES

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CONCLUSIONS

- The Shasta CUT&Tag workflow enables profiling of histone modifications at single-cell resolution for up to 1,500 cells per chip.
- Data generated using Shasta CUT&Tag allows for differentiation between epitherapy-treated and untreated cells.
- Gene expression analysis by Shasta Total RNA corroborates the findings of drug-induced epigenetic activation of *XIST*.

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