EpiFinder™ GenomePro

EPIGENCA

Monitoring Epigenomic Drug Response in Leukemia cells Using EpiFinder™ GenomePro

Background

The Intricate Landscape of Epigenetic Profiling

Epigenomic profiling holds immense potential for understanding gene regulation and disease mechanisms through the study of dynamic and reversible modifications such as DNA methylation and histone post-translational modifications (hPTMs). However, capturing the full complexity of the epigenome presents considerable technical challenges. Single modality approaches typically measure only one type of chromatin feature at a time, providing an incomplete picture of the layered regulatory landscape. Additionally, the high variability among cell states and populations complicates the generation of reproducible, quantitative data.

EpiFinder™ GenomePro: A Multiplexed Solution for Simultaneous Profiling of Multiple Epigenomic Marks

EpiFinder™ GenomePro leverages the MINUTE-ChIP method (1, 2) for multiplexed, quantitative profiling of histone modifications and DNA methylation across multiple samples in a single experiment. Early pooling of barcoded chromatin enables parallel processing under uniform conditions, improving reproducibility and enabling direct, quantitative comparisons between samples. A dedicated analysis pipeline converts raw sequencing data into normalized, scaled profiles for quantitative downstream interpretation (3). This highly sensitive and reproducible approach provides robust support for comprehensive and quantitative epigenomic studies.

The EpiFinder™ GenomePro Workflow

The EpiFinder™ GenomePro protocol streamlines epigenetic profiling into different key steps within 3 days: The workflow is summarized in Figure 1.

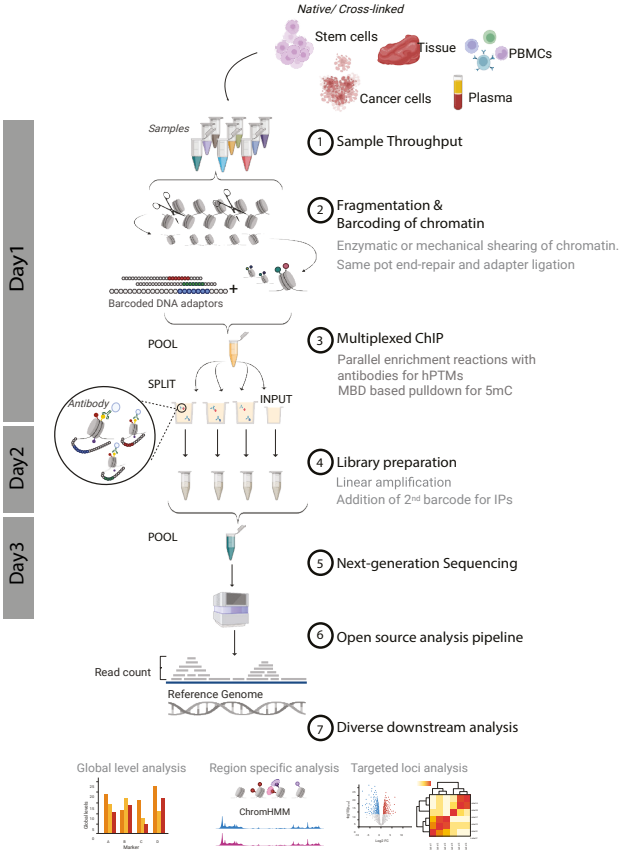

Figure 1. The schematic illustrates the workflow of EpiFinder™ GenomePro.

Definition

Input sample refers to a portion of the barcoded fragmented chromatin that is set aside before immunoprecipitation. It serves as a reference control to normalize and quantify enrichment of DNA fragments bound by the target protein or histone modification.

Case Study

Study Design

We applied our multiplexed EpiFinder™ workflow to profile chromatin changes induced by the histone deacetylase inhibitor Vorinostat (SAHA) in K562 cells. Cells were treated with four concentrations of SAHA (0.25, 0.5, 0.75, and 1 µM) plus a DMSO control in biological triplicate (i.e., 15 samples), for 72 hours (Fig. 2). Following treatment, native cell pellets were collected, micrococcal nuclease (MNase) digested to mono- to tri-nucleosome fragments and ligated with double-stranded DNA adaptors in a one-pot reaction. Barcoded samples were then pooled and aliquoted into individual ChIP reactions against five histone PTMs (H3K27me3, H3K4me3, H3K9me3, H3K9ac, H3K27ac), as well as RNA Polymerase II Ser2 phosphorylation (Pol II S2p) and DNA methylation (via Methyl Binding Domain (MBD)-based) (summarized in Table 1) (4, 5). ChIPed DNA was isolated and set up in sequential reactions of *in vitro* transcription, RNA 3′ adapter ligation, reverse transcription and PCR amplification to generate final libraries for each ChIP reaction. After quality assessment and concentration estimation, libraries were combined and sequenced on the Illumina NextSeq500 platform with paired-end settings.

The pool-split workflow reduces technical variation and enables robust comparative profiling across doses for each mark. Because Vorinostat is a well-characterized pan-HDAC inhibitor known to increase histone acetylation and thereby alter chromatin states, this system offers a good benchmark for sensitivity, linearity, and cross-mark concordance (6).

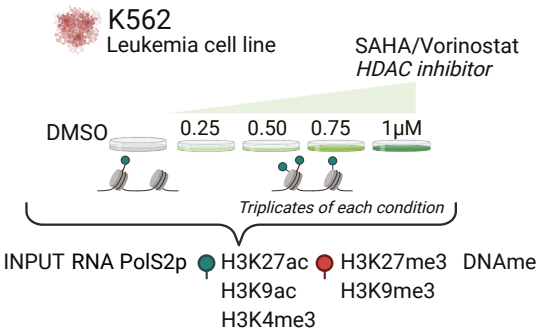

Figure 2. Graphical image illustrating the experimental setup for the K562 leukemia cell line with varying concentration of the histone deacetylase inhibitor Vorinostat/SAHA. K562 were studied for different histone modifications and methylation marks on the chromatin.

Note

The K562 leukemia cell line is ideal for epigenetic drug studies due to their dynamic chromatin landscape, high HDAC expression, and sensitivity to chromatin-modifying agents. Their transcriptional plasticity and compatibility with multi-omics profiling make them a robust model for evaluating epigenetic therapies, mechanisms of action, and resistance.

Table 1. Studied epigenetic marks in K562 leukemia cells lines and their role in gene expression.

Mark / Factor	Type	Associated Chromatin State	Role in Gene Expression
H3K27me3	Repressive methylation	Facultative heterochromatin	Silences genes; marks poised but inactive promoters
H3K4me3	Activating methylation	Active promoter	Promotes transcription initiation at TSS
H3K9me3	Repressive methylation	Constitutive heterochromatin	Maintains long-term gene silencing
H3K27ac	Activating acetylation	Enhancers and active promoters	Enhances transcriptional activity; marks active enhancers
H3K9ac	Activating acetylation	Open (euchromatin) and transcriptionally active regions	Enhances nucleosome accessibility, facilitates transcription initiation, and marks active promoters
RNA Polymerase II (S2p)	Transcriptional machinery Indicates elongation phase of transcription	Transcribed gene bodies	Indicates elongation phase of transcription
DNAme (5mC)	Repressive DNA methylation	Constitutive and facultative heterochromatin (CpG islands, repetitive DNA)	Stably silences gene expression; represses promoters, maintains genome stability, and prevents spurious transcription

Result

Validation of Loci-Specific Epigenetic Marks

From aggregated signal across all 15 samples (i.e. pooled coverage), we visualized genome browser tracks in Integrative Genomics Viewer (IGV) to confirm expected loci enrichments (Fig.3). Across marks, the profiles show clear separation of active vs repressive domains, and the alignment of peaks between replicates is high (low background "noise" in intervening regions). In the Histone Gene Cluster (e.g. H2BC8, H3C4) we observe strong accumulation of active marks (H3K4me3, H3K9ac, H3K27ac) and Pol II S2p signal, while repressive marks (H3K27me3, H3K9me3) and DNA methylation are depleted there. Conversely, in the HOX gene cluster (for example HOXD10, HOXD12), we see robust H3K27me3, H3K9me3, and DNAme signal, with minimal acetylation or Pol II, mirroring canonical repression patterns. In addition, a known repeat-rich region on chromosome 2 is strongly enriched for H3K9me3, consistent with heterochromatic repeat suppression.

The cross-mark consistency (i.e. anti-correlated patterns of acetylation vs methylation, and co-localization of Pol II with acetylation) indicates that the EpiFinder™ GenomePRO multiplex design preserves signal fidelity. The browser views, despite pooling, show sharp peaks and clear domain boundaries, suggesting robust signal-to--noise and good enrichment.

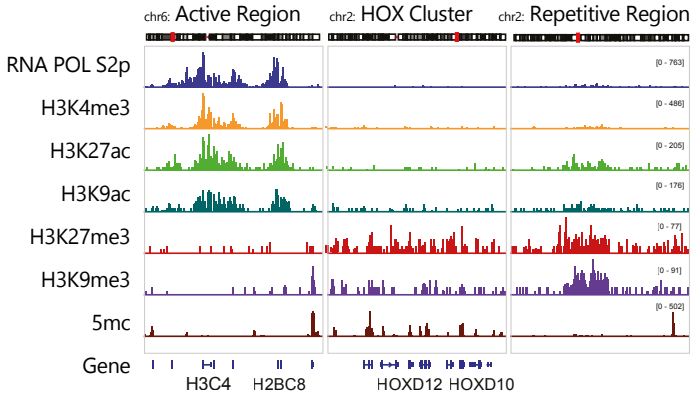

Figure 3. Genome browser (IGV) tracks displaying enrichment patterns for multiple epigenetic marks, including five histone modifications (H3K4me3, H3K27ac, H3K9ac, H3K27me3, H3K9me3), RNA Polymerase II (RNA Pol S2p), and DNA methylation (5mC), across three different genomic regions. Data represents combined signals from all samples treated with Vorinostat/SAHA (an HDAC inhibitor) and DMSO control.

Figure 3, Panel 1:

Histone gene clusters (ex: H2BC8 and H3C4) encode the core histone proteins (H2A, H2B, H3, H4) that package DNA into nucleosomes. These genes are constitutively expressed to maintain chromatin structure and support ongoing DNA replication and transcription. Their promoters are typically enriched for active chromatin marks, making them useful reference loci for detecting active histone modifications.

Figure 3, Panel 2:

HOX genes control body plan and developmental patterning by encoding transcription factors that regulate cell fate. In differentiated cells, many HOX loci are repressed through Polycomb group proteins, which deposit the histone modification H3K27me3 and establish long--term silencing. These regions serve as canonical examples of Polycomb-mediated repression and are expected to show strong enrichment for repressive histone marks.

Figure 3, Panel 3:

Large stretches of pericentromeric and sub telomeric repeats on chromosome 2 are typically silenced to preserve genome stability. These repetitive elements are marked by H3K9me3, a heterochromatin--associated modification. Their enrichment provides a clear example of constitutive heterochromatin and serves as an internal control for repressive chromatin profiling.

Treatment Effect on Epigenetic Marks

In the dose–response dimension, we assessed how each mark's signal (shifts across increasing SAHA concentrations relative to DMSO control (Fig.4). For each epigenetic mark, total mapped read counts per sample were normalized to the respective input read counts, essentially correcting for uneven barcode representation in the input. The resulting input normalized mapped read count (INRC) serves as a quantitative measure of the abundance of ChIP epitope.

Notably, H3K27ac and H3K9ac display clear dose--dependent increases in enrichment. This is an expected outcome since the inhibition of histone deacetylases will lead to increased acetylation. In contrast, H3K27me3 remains unchanged with increasing doses of SAHA. This differential sensitivity demonstrates that the assay with HADCi can accurately reflect a biologically meaningful chromatin remodeling (here, induced acetylation) and detect specific responses in cells.

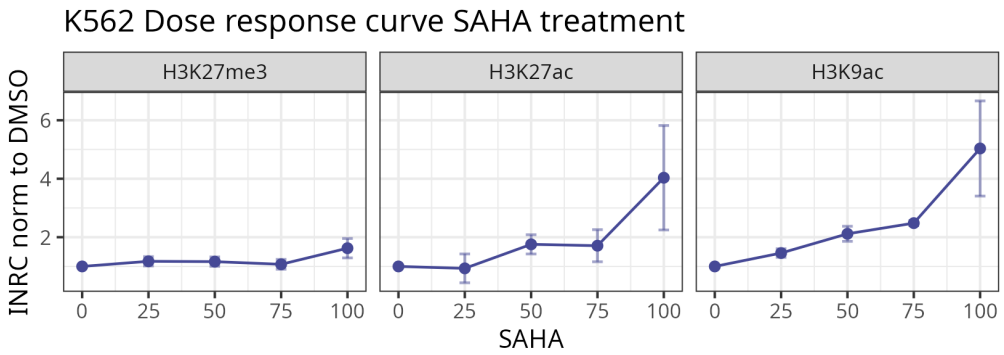

Figure 4. Dose response curve showing change in H3K27me3, H3K27ac and H3K9ac levels across increasing concentration of Vorinostat/SAHA (HDAC inhibitor). Input normalized read count (INRC) was calculated for each concentration, relative to the DSMSO control. Each point corresponds to the average INRC value of triplicate per condition with error bars showing standard deviation.

Summary Points

- Specificity: Demonstrates that Vorinostat selectively modulates acetylation without disrupting other epigenetic marks.
- Mechanistic Validation: Supports the use of acetylation marks as reliable readouts for HDACi activity.
- Epigenetic Profiling Utility: Confirms that EpiFinder™ GenomePro can sensitively detect drug-induced chromatin changes.

Conclusion

This study illustrates that our high-throughput, multiplexed workflow robustly detects chromatin signatures across multiple histone modifications, Pol II, and DNA methylation in a pooled-dose treatment paradigm, with clean signal separation at canonical loci and discernible dose responses for dynamic marks. The SAHA experiment recapitulates known biology: dose-dependent increases in histone acetylation (especially H3K27ac, H3K9ac), while trimethylation marks such as H3K27me3 remain largely unchanged over the treatment window.

Summary/Outlook

EpiFinder™ GenomePro proves to be a powerful platform for studying epigenetic modifications, offering deeper insight into cross-mark relationships (for example, correlating acetylation gain with methyl mark retention or displacement), the interplay between promoter acetylation and gene activation via Pol II dynamics, or coupling with matched transcriptome (RNA-seq) to link chromatin state changes with expression outcome. In a drug discovery context, this platform could be deployed for epigenetic screening: profiling multiple doses across multiple compounds and assessing their chromatin "signatures" in one multiplexed experiment. Moreover, the same framework could be extended to additional histone PTMs (e.g. H3K36me3, H3K27me1/2, H4 acetylation) to broaden mechanistic coverage.

References

1) Kumar, B., Navarro, C., Yung, P.Y.K. et al. Multiplexed chromatin immunoprecipitation sequencing for quantitative study of histone modifications and chromatin factors. *Nat Protoc* **20**, 779–809 (2025). https://doi.org/10.1038/s41596-024-01058-z

2) Kumar B, Elsässer SJ. Quantitative Multiplexed ChIP Reveals Global Alterations that Shape Promoter Bivalency in Ground State Embryonic Stem Cells. Cell Rep. 2019 Sep 17;28(12):3274-3284.e5. doi: 10.1016/j.celrep.2019.08.046. PMID: 31533047; PMCID: PMC6859498.

3) Navarro C, Martin M, Elsässer SJ. minute: A MINUTE-ChIP data analysis workflow. bioRxiv. 2022 Mar 17:2022.03.14.484318. doi: 10.1101/2022.03.14.484318. PMCID: PMC8949155.

4) Millán-Zambrano, G., Burton, A., Bannister, A.J. et al. Histone post-translational modifications — cause and consequence of genome function. *Nat Rev Genet* **23**, 563–580 (2022). https://doi.org/10.1038/s41576-022-00468-7

5) Mattei AL, Bailly N, Meissner A. DNA methylation: a historical perspective. Trends Genet. 2022 Jul;38(7):676-707. doi: 10.1016/j.tig.2022.04.007. Epub 2022 Apr 30. PMID: 35455684.

6) Krautkramer KA, Reiter L, Denu JM, Dowell JA. Quantification of SAHA-Dependent Changes in Histone Modifications Using Data-Independent Acquisition Mass Spectrometry. J Proteome Res. 2015 Aug 7;14(8):3252-62. doi: 10.1021/acs.jproteome.5b00245. Epub 2015 Jul 13. PMID: 26120868; PMCID: PMC4564294.