Data Descriptor: ChIP-seq and ChIP-exo profiling of Pol II, H2A.Z, and H3K4me3 in human K562 cells

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The human K562 chronic myeloid leukemia cell line has long served as an experimental paradigm for functional genomic studies. To systematically and functionally annotate the human genome, the ENCODE consortium generated hundreds of functional genomic data sets, such as chromatin immunoprecipitation coupled to sequencing (ChIP-seq). While ChIP-seq analyses have provided tremendous insights into gene regulation, spatiotemporal insights were limited by a resolution of several hundred base pairs. ChIP-exonuclease (ChIP-exo) is a refined version of ChIP-seq that overcomes this limitation by providing higher precision mapping of protein-DNA interactions. To study the interplay of transcription initiation and chromatin, we profiled the genome-wide locations for RNA polymerase II (Pol II), the histone variant H2A.Z, and the histone modification H3K4me3 using ChIP-seq and ChIP-exo. In this Data Descriptor, we present detailed information on parallel experimental design, data generation, quality control analysis, and data validation. We discuss how these data lay the foundation for future analysis to understand the relationship between the occupancy of Pol II and nucleosome positions at near base pair resolution.

| Design Type(s)       | parallel group design |
|----------------------|-----------------------|
| Measurement Type(s)  | transcription factor binding site identification |
| Technology Type(s)   | ChIP-seq assay ● chromatin immunoprecipitation with exonuclease sequencing assay |
| Factor Type(s)       | biological replicate  |
| Sample Characteristic(s) | immortal human cell line cell |

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Background & Summary

Control of eukaryotic transcription patterns involves the interplay of RNA polymerase II (Pol II) and chromatin. In metazoans, once Pol II initiates transcription, it rapidly transitions to a regulated paused state, 30–50 base pairs (bp) downstream of the transcription start site (TSS)\(^1\). In this position, Pol II is juxtaposed with the first nucleosome downstream of the TSS\(^2\,3\). The +1 nucleosome is specifically enriched with the histone variant H2A.Z and tri-methylation of the fourth N-terminal lysine on the histone H3 tail (H3K4me3). It has been known for several decades that Pol II must overcome nucleosomal obstacles during transcription\(^4\). However, questions remain regarding the molecular mechanisms underlying how chromatin regulates Pol II activity, and vice versa.

Since functional genomic approaches often require tens of millions of cells per assay, immortalized mammalian cell lines are frequently used in these studies. Due to its facile growth characteristics and its designation as an ENCODE tier 1 cell line, K562 cells are one of the most commonly used mammalian cell lines. The K562 cell line was originally established from a female patient with chronic myeloid leukemia\(^5\). K562 cells are considered erythroleukemic, displaying characteristics of undifferentiated granulocytes and erythrocytes\(^6\). In the presence of specific chemical inducers, K562 cells will differentiate along the erythroid lineage and upregulate globin expression\(^7\)–\(^9\).

As functional genomic technologies improve, they present new opportunities to address key biological questions. Chromatin immunoprecipitation coupled to high throughput sequencing (ChIP-seq) is a powerful tool to study mechanisms of gene regulation by selectively enriching for DNA fragments that interact with a given protein in living cells. Briefly, in vivo protein-DNA interactions are preserved through covalent linkage as a result of formaldehyde treatment. Cells are lysed, the nuclear fraction is retained, and the chromatin is fragmented by sonication to 100–500 bp. DNA fragments interacting with the protein of interest are enriched by ChIP, and a library is prepared by adding sequencing adapters according to manufacturer’s instructions. Genomic regions that interact with the protein of interest are deduced by sequencing from the sonication borders, which are typically several hundreds of base pairs away from the protein-DNA crosslinked interaction site.

A more recently developed technology, called ChIP-exo, improves upon ChIP-seq by providing near base pair mapping resolution for protein-DNA interactions. The key innovation of the ChIP-exo methodology is the incorporation of lambda exonuclease digestion in the library preparation workflow to effectively footprint the left and right 5’ DNA borders of the protein-DNA crosslink site. Thus, rather than sequencing from the distal sonication borders as in ChIP-seq, ChIP-exo enriched DNA fragments are sequenced from the left and right 5’ DNA borders of the protein-DNA crosslink site. The precision of the resulting data can be leveraged to provide unique and ultra-high resolution insights into the functional organization of the genome. Given its high base pair resolution, ChIP-exo is uniquely capable of spatially resolving divergent, initiating, paused, and elongating RNA polymerase II on a genome-wide scale. For example, our related work used Pol II ChIP-exo analysis in K562 cells to show that divergent transcription at promoters arises from distinct, resolvable pre-initiation complexes (PICs)\(^10\). Reanalysis of

| ChIP target | Antibody            | Assay   | Replicate | Sample ID      | Total Mapped Reads | Uniquely Mapped Reads | Unique Mapping Rate |
|-------------|---------------------|---------|-----------|----------------|---------------------|-----------------------|---------------------|
| Pol2        | sc899 (Santa Cruz)  | ChIP-seq| 1         | SAMN07546015  | 28,247,807          | 24,603,174            | 87%                 |
|             |                     | ChIP-seq| 2         | SAMN07546016  | 94,976,221          | 85,443,543            | 90%                 |
|             | TOTAL               |         |           |                | 123,224,028         | 110,046,717           | 90%                 |
|             | ChIP-exo            | 1       | SAMN07546015 | 38,286,067 | 32,989,473         | 86%                  |
|             |                     | 2       | SAMN07546016 | 56,147,982 | 50,109,500         | 89%                  |
|             | TOTAL               |         |           |                | 94,434,049          | 83,098,973           | 89%                 |
| H2A.Z       | 07-594 (EMD Milipore)| ChIP-seq| 1         | SAMN07546015  | 23,004,351          | 20,145,444            | 88%                 |
|             |                     | ChIP-seq| 2         | SAMN07546016  | 35,928,019          | 32,559,626            | 91%                 |
|             | TOTAL               |         |           |                | 58,932,370          | 52,705,070           | 90%                 |
|             | ChIP-exo            | 1       | SAMN07546015 | 27,043,543 | 23,726,092         | 88%                  |
|             |                     | 2       | SAMN07546016 | 64,925,557 | 58,836,047         | 91%                  |
|             | TOTAL               |         |           |                | 91,969,100          | 82,662,139           | 91%                 |
| H3K4me3     | ab8580 (Abcam)      | ChIP-seq| 1         | SAMN07546015  | 57,472,144          | 52,954,309            | 92%                 |
|             |                     | ChIP-seq| 2         | SAMN07546016  | 56,431,834          | 52,528,142            | 93%                 |
|             | TOTAL               |         |           |                | 113,903,978         | 105,482,451           | 92%                 |
|             | ChIP-exo            | 1       | SAMN07546015 | 19,935,227 | 16,778,098         | 84%                  |
|             |                     | 2       | SAMN07546016 | 32,325,622 | 47,444,745         | 91%                  |
|             | TOTAL               |         |           |                | 72,260,849          | 64,222,843            | 91%                 |

Table 1. Sequencing read alignment statistics for ChIP-seq and ChIP-exo.
this data by Lis and colleagues showed that enhancers and promoters share a unified transcription initiation architecture. Sandelin and colleagues repurposed our Pol II ChIP-exo data to provide corroborative evidence for alternative transcription initiation within closely spaced promoters. Finally, reanalysis by Lukatsky and colleagues found a DNA triplicate code linked to PIC positioning at promoters.

In this Data Descriptor, we extend the value of our previous Pol II ChIP-exo data by generating 12 new ChIP-seq and ChIP-exo data sets for Pol II, H2A.Z, and H3K4me3 in K562 cells. ChIP-exo mapping of Pol II, a histone variant, and a histone modification should enable other investigators to use these data sets for their own research to further understand the detailed interplay of Pol II and chromatin. Further, paired libraries generated side-by-side should enable direct comparisons between the quality of ChIP-seq and ChIP-exo mapping genome-wide. On average, 42 million uniquely aligned reads were generated for each ChIP-seq and ChIP-exo data set (Table 1). To facilitate interpretation of these data, we provide detailed information on experimental design (Fig. 1), sequence quality control analyses (Fig. 2), and biological validation (Fig. 3).

**Methods**

**Tissue culture**

Human chronic myelogenous leukemia cells (K562, ATCC) were maintained at 37 °C in 5% CO₂ between 0.1–1 million cells/ml in DMEM (Dulbecco’s Modified Eagle Media) containing 10% bovine calf serum and 1% Penicillin/Streptomycin.

**ChIP-seq and ChIP-exo library preparation**

ChIP-exo was performed as previously described with chromatin extracted from 50 million cells, ProteinG MagSepharose resin (GE Healthcare), and 5 ug of antibody directed against RNA polymerase II,
H2A.Z, or H3K4me3, (Santa Cruz sc899, EMD Millipore 07-594, or Abcam ab8580, respectively). For each biological replicate, ChIP-seq and ChIP-exo libraries were prepared using the same starting sonicated nuclear extract. Importantly, this controls for more direct comparisons ChIP-seq and ChIP-exo for each antibody used. Libraries were sequenced using an Illumina NextSeq500 sequencer as single-end reads 50 or 75 nucleotides in length (Table 1).

Sequence read alignment and quality control
The base call quality for each sequenced read was assessed using the FastQC program (bioinformatics.babraham.ac.uk/projects/fastqc/) (Fig. 2a and Supplementary Figs. 1–2). Sequence reads (fastq files) were aligned to the human hg19 reference genome build using BWA-MEM algorithm with default parameters. The resulting bam files were first sorted using the Samtools Sort function, and then bam index files were generated using the Samtools Index function. The purpose of bam index files is to enable viewing of raw sequencing data in a genome browser. Next, genome-wide read coverage and enrichment were assessed using deepTOOLS fingerprint plots (Fig. 2b and Supplementary Fig. 3).

Biological validation
To estimate variance across biological replicates, the Pearson correlation coefficient for pairwise gene Reads Per Kilobase of genome per Million reads (RPKM) was computed (Fig. 2c, Supplementary Fig. 4) using the HOMER suite (Hypergeometric Optimization of Motif EnRichment). Briefly, bam files were converted to tag directories using the makeTagDirectory function with the –genome, –checkGC, and

Figure 2. Quality control, enrichment analysis, and reproducibility for ChIP-seq and ChIP-exo data. (a) Box-plot distribution of base quality scores are shown for Pol II ChIP-exo replicate 1. A score greater than 30 (green region) indicates a high confidence base call. (b) ChIP-enrichment analysis plot that displays the cumulative percent of total reads found in a given percent of the mappable human genome. No ChIP enrichment would result in a diagonal trace. (c) Scatter plot correlation analysis for Pol II ChIP-exo biological replicates as measured by the Spearman correlation coefficient R-values (upper right corner).
To quantify and normalize tags within gene body regions to RPKM, the analyzeRepeats function was used with the --rpkm and --d options (Data Citation 1). ChAsE (Chromatin Analysis and Exploration) visualization suite was used to display the distribution of Pol II, H2A.Z, and H3K4me3 relative to the TSS (Fig. 3a and b, Supplementary Fig. 6). Raw sequencing tags were binned, smoothed, and RPKM computed using the deepTOOLS genomeCoverage tool (20 bp bin, 100 bp sliding window) (Fig. 3c). Smoothed RPKM signal was visualized with Integrative Genomics Viewer (IGV) (Fig. 3c).

Code availability
Below is a list of software used in this study.

15BWA-MEM v0.7.13
16Samtools v1.3.1
17FastQC v0.11.2 (bioinformatics.babraham.ac.uk/projects/fastqc/)
ChIP enrichment (Supplementary Fig. 3). Theoretically, complete genome coverage with no enrichment ChIP-exo data. Fingerprint plots for other replicates showed similar patterns of genome coverage and enriched with 60% of all uniquely aligned reads (y-axis, 100-40), suggesting strong enrichment Pol II of all uniquely aligned reads, respectively. Together, these values re

From the axes, the corresponding values on the x- and y-axes denote the percent of genome and the percent trace indicates the extent of ChIP enrichment. Given a point along the trace that is the point of intersection fingerprint plots, a rightward deflection ChIP enrichment.

Raw sequence quality control analyses
To assess the quality of the raw sequencing data sets, base call scores were analyzed using the FastQC program and displayed as a box plot distribution at each base position (Fig. 2a and Supplementary Figs. 1–2). The average base quality score for all 12 ChIP data sets in the present study fell within the high confidence range (base quality score of 30–40, green region).

Raw sequence reads were aligned to the hg19 build of the human genome. On average, 46 million total aligned reads were generated for each ChIP-seq and ChIP-exo data set (Table 1), ranging from 20-95 million reads. Because of the ambiguity of reads that align to multiple locations throughout the genome, we only retain uniquely aligned reads for subsequent analyses. On average, 42 million uniquely aligned reads were obtained per data set, representing unique alignment rates between 84-93%.

Two critical questions for assessing ChIP sequencing data quality are: 1) how much of the genome is represented by a given experiment? and 2) to what extent did the ChIP assay enrich for specific regions of the genome? Typically, high genome coverage and strong ChIP enrichment are desirable in ChIP experiments.

To determine genome coverage and ChIP enrichment simultaneously, we used the deepTOOLS suite to perform a fingerprint analysis (Fig. 2b). In the case of Pol II ChIP-exo (Fig. 2b), the fingerprint plot trace intersects the x-axis at 15, indicating 85% genome coverage. In fingerprint plots, a rightward deflection of the trace indicates the extent of ChIP enrichment. Given a point along the trace that is the point of intersection from the axes, the corresponding values on the x- and y-axes denote the percent of genome and the percent of all uniquely aligned reads, respectively. Together, these values reflect ChIP enrichment.

For example, the Pol II ChIP-exo fingerprint trace reveals that 20% of the genome (x-axis, 100-80) is enriched with 60% of all uniquely aligned reads (y-axis, 100-40), suggesting strong enrichment Pol II ChIP-exo data. Fingerprint plots for other replicates showed similar patterns of genome coverage and ChIP enrichment (Supplementary Fig. 3). Theoretically, complete genome coverage with no enrichment would be result in a trace with a slope equal to one that intersects the origin (eg: whole genome sequencing wherein 50% of all aligned reads).

Biological validation
After verifying the quality of the raw sequencing data, we next sought to provide evidence of biological validity for the data. First, we determined the extent to which biological replicates were reproducible using correlation scatter plots (Fig. 2c). For each gene, the RPKM was computed using the HOMER suite (Data Citation 1). Pearson correlation coefficients (R-values) were computed for pairwise correlation plots of gene RPKMs across biological replicates. For example, biological replicates for Pol II ChIP-exo analysis displayed an R-value of 0.96, indicating high reproducibility (Fig. 2c). Correlation analysis of other data resulted in positive R-values between 0.56 and 0.99 (Supplementary Fig. 4). Similarity across ChIP-seq and ChIP-exo for each factor were assessed by correlation analysis between merged ChIP-exo and ChIP-seq data sets, which displayed R-values between 0.86 and 0.99 (Supplementary Fig. 5).

Given that certain transcription factors operate at a consistent distance from TSSs, analyzing global patterns of ChIP signal relative to TSSs is a useful method to assess biological validation. It is well established
that once Pol II initiates transcription of genes in metazoans, Pol II moves into a stable paused state 30–50 bp downstream of the TSS. Likewise, H2A.Z and H3K4me3 are consistently incorporated primarily into the +1 nucleosome of actively transcribed genes. Thus, to examine global patterns of ChIP enrichment, the Chromatin Analysis and Exploration (ChaSE) heatmap tool was used to align ChIP signal merged from both biological replicates to TSSs (Fig. 3a, sorted by max peak; and Supplementary, Fig. 6, sorted by max peak position). Quantification of signal density relative to TSSs is displayed as a composite plot below each heatmap (Fig. 3b). As expected, Pol II ChIP signal was sharply enriched just downstream of the TSS at the pause site for both ChIP-seq and ChIP-exo data. H2A.Z and H3K4me3 signals were broadly enriched up- and downstream of the TSS, consistent with the -1 and +1 nucleosome positions. To examine individual examples of global patterns, RPKM normalized tracks for ChIP signal were displayed using the Integrative Genome Viewer (IGV). The distribution of ChIP signal at a histone cluster and the RPS12 gene recapitulated the global patterns of Pol II, H2A.Z, and H3K4me4 (Fig. 3c). Taken together, we conclude that the data presented in this Data Descriptor represent high quality next generation sequencing data that are biologically valid, and should be useful to future studies that seek to understand the interplay of Pol II and chromatin in high resolution on a global scale.

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Data Citations
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Author Contributions
Z.H. and A.P. conducted bioinformatics data analysis, prepared figures, and provided comments on the manuscript. B.V. designed and performed experiments, conceived bioinformatic analyses, and wrote the manuscript.

Additional Information
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