Data in Brief

# Changes in nucleosome occupancy occur in a chromosome specific manner

Brittany S. Sexton, Brooke R. Druliner, Denis Avey, Fanxiu Zhu, Jonathan H. Dennis *

Department of Biological Science, The Florida State University, Tallahassee, FL 32306-4295, USA

A B S T R A C T

In the eukaryotic nucleus, DNA is packaged into chromatin. The fundamental subunit of chromatin is the nucleosome, DNA is wrapped 1.6 times around a histone octamer core. Nuclear processes in eukaryotes are impacted by whether regulatory DNA is occupied by nucleosomes. We used microarrays to measure nucleosome occupancy in human cells post-Kaposi's sarcoma-associated herpesvirus (KSHV) reactivation at hundreds of immunity-related loci. The detailed analysis of these technologies can be found in recent publications from our lab [1,3]. We found that nucleosome redistributions displayed chromosome specific nucleosome occupancy. This resource can be used to map nucleosome distributions in a variety of biological contexts.

© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

| Specifications | |
|---|---|
| Organism/cell line/tissue | iSLK.rKSHV219 cell lines |
| Sex | Male |
| Sequencer or array type | NimbleGen's 12-plex and HD2 design were utilized for the custom DNA microarray 100319_HG19_Immunity_JD_CGH Array and were used in this study. We customized the microarray to contain 1 kb upstream and downstream of the transcription start sites of hundreds of immunity-related loci. |
| Data format | Pair files and .gff files (raw and processed). |
| Experimental factors | KSHV was reactivated in iSLK.rKSHV219 cell lines with doxycycline at a final concentration of 0.2 µg/ml. |
| Experimental features | Measured nucleosome distribution at 0, 6, 12, 24, and 48 h post-KSHV reactivation at hundred of immunity-related loci using a custom tiling microarray. |
| Consent | NA |
| Sample source location | NA |

## Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52453.

* Corresponding author.

## Experimental design, materials and methods

*Cell culture and KSHV reactivation in iSLK.rKSHV219 cells*

rKSHV.219 virus is latently infected in iSLK.rKSHV219 cell line, derived from iSLK cell line, as previously described by [4]. Clone 10 of iSLK.219 cells was grown in culture according to [2]. rKSHV.219 cells were seeded at $5 \times 10$ cells into 150-mm dish (one day prior to induction). For induction of KSHV reactivation, the previous medium is replaced with 1% FBS DMEM media containing a final concentration of 0.2 µg/ml doxycycline. KSHV reactivation was induced at 0, 6, 12, 24, and 48 h. The 0 h sample was treated identically to other timepoints, however doxycycline was not added. Two independent biological replicates for each timepoint in iSLK.rKSHV219 cell lines were processed and behaved similar for the nucleosome distribution assay [3].

*Post-KSHV reactivation cell harvest and nucleus purification*

Following doxycycline induction of KSHV reactivation, iSLK.rKSHV219 cells were harvested at 0, 6, 12, 24, and 48 h at $2.5 \times 10^7$ cells. Media was aspirated and then 1% formaldehyde in PBS was incubated with the cells for 10 min at room temperature, to cross-link the chromatin structure. 125 mM glycine was then added to stop the formaldehyde cross-linking reaction. Nucleus isolation buffer, 0.3 M sucrose, 2 mM MgOAc$_2$, 1 mM CaCl$_2$, 1% Nonidet P-40, 10 mM HEPES, and pH 7.8 were then added to iSLK.rKSHV219 cross-linked cells. The cells were scraped and placed within a 50 ml conical tube, and the nuclei were isolated by centrifugation at 1000 ×*g* for 5 min at 4 °C.
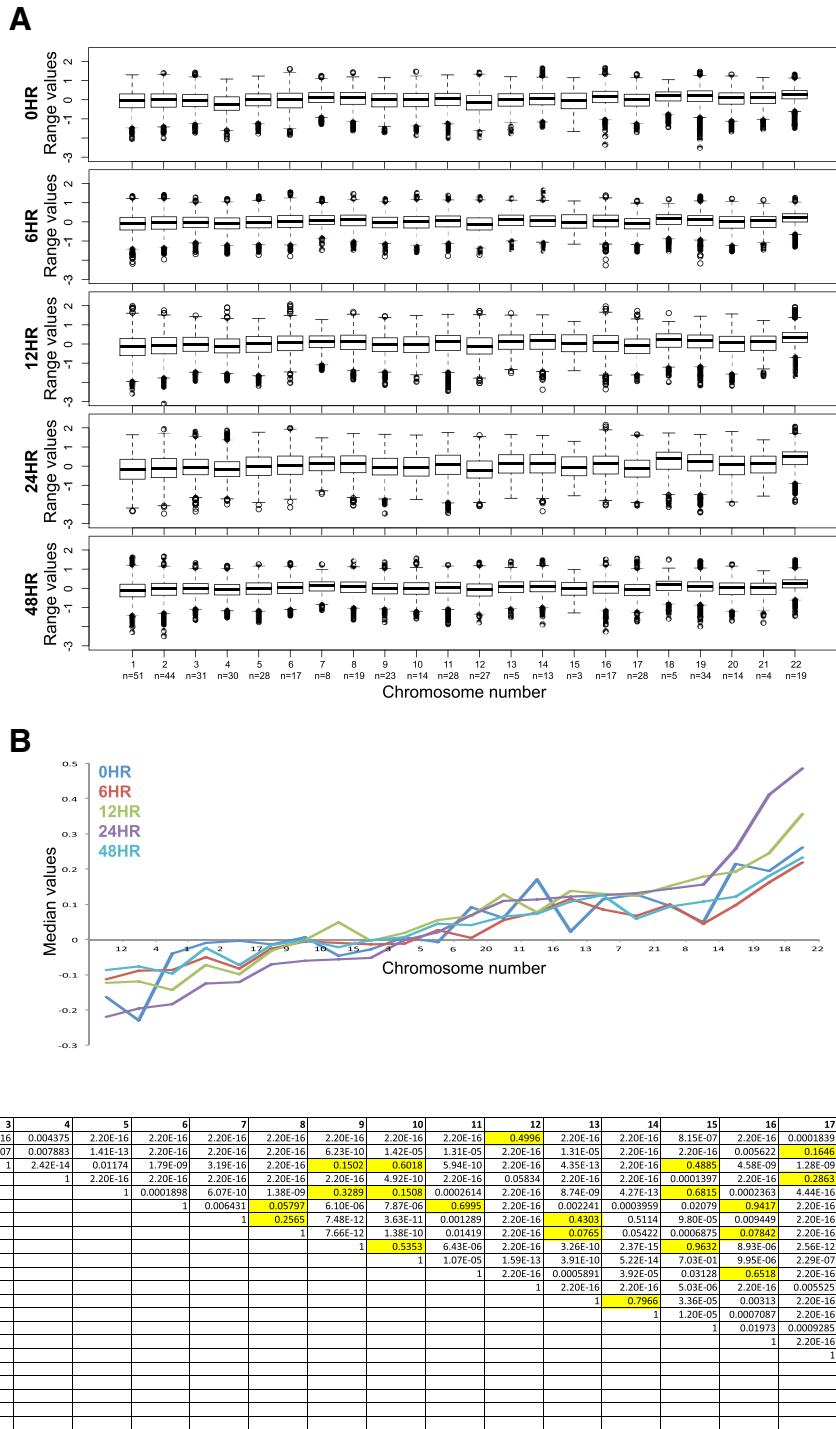
**Fig. 1.** Nucleosome position and occupancy measurements at each chromosome during the reactivation of KSHV. (A) Boxplot representation for ranges of nucleosome positioning values of all loci sorted by chromosome at all timepoints tested: 0 h, 6 h, 12 h, 24 h, and 48 h. Number of loci measured per chromosome (n) is noted below each boxplot. (B) Median nucleosome occupancy values for all timepoints tested, sorted in ascending order by median occupancy at 24 h timepoint. (C) Statistical analysis of the nucleosome distribution changes at the 24 hour timepoint on a per chromosome basis. P-values derived from a t-statistic are shown for the pairwise comparison of the range of values between all chromosomes.

*MNase digestion and purification of mononucleosomal protected DNA*

Micrococcal nuclease, MNase, is a internucleosomal cleavage reagent, which does not cleave the DNA protected by a nucleosome. iSLK.rKSHV219 nuclei were digested with a titration of MNase: 4 U/ml, 2 U/ml, 1 U/ml, and 0.5 U/ml (Worthington Biochemical Corp.) in MNase cleavage buffer (5 mM KCl, 4 mM MgCl$_2$, 1 mM CaCl$_2$, 12.5% glycerol, and 50 mM Tris–Cl (pH 7.4)). Nuclei were incubated at 37 °C for 5 min for MNase digestion. These reactions were stopped, following incubation, with 50 mM EDTA. A titration of MNase concentrations was utilized to ensure nucleosomes that are more and less readily cleaved are equally represented. The nuclei, following MNase digestion, were treated with 0.2 μg/μl proteinase K and 1% sodium dodecyl sulfate. The DNA-protein crosslinks are then reversed by 60 °C overnight incubation.

MNase digested DNA for all samples was resolved on a 2% agarose gel, and mononucleosomally protected DNA, ~150 bp, was isolated by

gel excision. Following gel excision, the mononucleosomally protected DNA was purified by electroelution, and for each sample's MNase titration the mononucleosomally protected DNA was combined. The mononucleosomally protected DNA was further purified with phenol–chloroform extraction and precipitated with alcohol. The precipitated DNA was then dissolved with TE, 10 mM Tris–Cl, pH 8.0, and 0.1 mM EDTA. The reference used in the nucleosome distribution assay was iSLK.rKSHV219 bare genomic DNA. iSLK.rKSHV219 cells were resuspended in 0.2-μg/μl proteinase K and 1% SDS solution and incubated at 55 °C overnight for bare genomic DNA isolation.

*Microarray design and processing*

We utilized a high-resolution DNA microarray, which we customized to contain 1 kb upstream and downstream of the transcription start sites of 462 immunity-related genes. NimbleGen's 12-plex and HD2 design were utilized for the custom DNA microarray 100319_HG19_Immunity_JD_CGH Array. Sequences were repeat-masked around the TSSs, and only probes containing unique sequences for both the forward and reverse DNA strands were printed on the microarray. Oligonucleotide probes, sized at 60 bp, were tiled on each subarray with a mean spacing of 47 bp overlap. Approximately 180 probes of spacing of 12.5 bp were printed on the microarray for each locus. The samples were fluorescently labeled: nucleosomally protected DNA with Cy3 and bare genomic DNA (reference) with Cy5. Samples were hybridized to the DNA microarray according to NimbleGen's instructions (http://www.nimblegen.com/technology/index.html).

*Data processing and analysis*

Microarrays were scanned on the NimbleGen MS200 Microarray Scanner at 2 μm resolution. The resulting .tiff files were used for microarray data feature extraction, primary data analysis and generation of initial data files using the NimbleGen DEVA software version 1.2 CGH workflow. Specifically, LOESS Spatial Correction was applied to the raw data; Background Correction was used to determine and adjust the signal data for noise using specifically designed background control probes; and Qspline Normalization was applied to compensate for inherent differences in Cy3 and Cy5 signal. The ratio between nucleosomal (Cy3) and bare genomic Cy5 signals was then calculated and stored as a General Feature Format, .gff file for subsequent analysis. Subsequent analysis was performed using a software developed in our laboratory DennisLab.R, which runs in the R environment for statistical computing. DennisLab.R is freely available at www.chromatin.bio.fsu.edu. DennisLab.R was used to generate summary statistics on the range of nucleosome distribution values for each individual chromosome. Finally, we analyzed the nucleosome distribution changes at the 24 hour timepoint on a per chromosome basis, by calculating the t-statistic and P-value (assuming unequal variance) for the pairwise comparison of the range of values between all chromosomes.

*Nucleosome occupancy changes occur in a chromosome specific manner*

In our previous work we demonstrated that nucleosome redistributions are widespread, transient, and DNA-directed [1,3]. We next wanted to determine if nucleosome redistributions showed chromosome specific changes in a time dependent manner. We first calculated the range of nucleosome occupancy values for all loci studied on a per chromosome basis for each post-KSHV reactivation timepoint (Fig. 1A). The 24 hour timepoint consistently showed the greatest range of nucleosome occupancy values. These results comport with our previous observation that the greatest positioning occurs at the 24 hour timepoint, however do not indicate chromosome specificity with respect to increased positioning.

We next wanted to investigate if the nucleosome redistribution changes at the 24 hour timepoint reflected increased or decreased nucleosomal occupancy. We calculated the median nucleosome occupancy values on each chromosome for all of the loci measured. We discovered that nucleosome occupancy values change in a chromosome specific manner (Fig. 1B). Chromosomes 18, 19, and 22 show the greatest median nucleosome occupancy values at the 24 hour timepoint. Conversely, chromosomes 1, 2, 9, 10, 12, 15, and 17 show the lower median nucleosome occupancy values at the 24 hour timepoint.

We were next interested in calculating the statistical significance of the chromosome specific nucleosome occupancy changes that we had identified at the 24 hour timepoint. Most of the ranges of nucleosome occupancy values are not statistically significantly different from one another (Fig. 1C, yellow highlights). Two chromosomes, 18 and 22, are statistically different from all other chromosomes. These two chromosomes show clear increases in the nucleosome occupancy relative to the other chromosomes. At the other end of the scale, chromosomes 1 and 12 are statistically indistinguishable, yet different from all other chromosomes. Chromosomes 1 and 12 have the broadest range of values (Fig. 1A) and a lower median (Fig. 1C) suggesting an overall decrease in nucleosomal occupancy in these chromosomes. Thus, the trend observed in Figs. 1A and B are statistically significant. These results demonstrate that while nucleosomal positioning remains relatively constant between chromosomes at each timepoint, nucleosomal occupancy changes in a chromosome specific manner.

### References

[1] B.R. Druliner, J.A. Fincher, B.S. Sexton, D.L. Vera, M. Roche, S. Lyle, J.H. Dennis, Chromatin patterns associated with lung adenocarcinoma progression. Cell Cycle 12 (2013) 1536–1543.
[2] J. Myoung, D. Ganem, Generation of a doxycycline-inducible KSHV producer cell line of endothelial origin: maintenance of tight latency with efficient reactivation upon induction. J. Virol. Methods 174 (2011) 12–21.
[3] B.S. Sexton, D. Avey, B.R. Druliner, J.A. Fincher, D.L. Vera, D.J. Grau, M.L. Borowsky, S. Gupta, S.B. Girimurugan, E. Chicken, J. Zhang, W.S. Noble, F. Zhu, R.E. Kingston, J.H. Dennis, The spring-loaded genome: nucleosome redistributions are widespread, transient, and DNA-directed. Genome Res. 24 (2) (Feb. 2014) 251–259.
[4] J. Vieira, P.M. O'Hearn, Use of the red fluorescent protein as a marker of Kaposi's sarcoma-associated herpesvirus lytic gene expression. Virology 325 (2004) 225–240.