Epidaurus: aggregation and integration analysis of prostate cancer epigenome

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ABSTRACT

Integrative analyses of epigenetic data promise a deeper understanding of the epigenome. Epidaurus is a bioinformatics tool used to effectively reveal inter-dataset relevance and differences through data aggregation, integration and visualization. In this study, we demonstrated the utility of Epidaurus in validating hypotheses and generating novel biological insights. In particular, we described the use of Epidaurus to (i) integrate epigenetic data from prostate cancer cell lines to validate the activation function of EZH2 in castration-resistant prostate cancer and to (ii) study the mechanism of androgen receptor (AR) binding deregulation induced by the knockdown of FOXA1. We found that EZH2’s noncanonical activation function was reaffirmed by its association with active histone markers and the lack of association with repressive markers. More importantly, we revealed that the binding of AR was selectively reprogrammed to promoter regions, leading to the up-regulation of hundreds of cancer-associated genes including EGFR. The prebuilt epigenetic dataset from commonly used cell lines (LNCaP, VCaP, LNCaP-Abl, MCF7, GM12878, K562, HeLa-S3, A549, HePG2) makes Epidaurus a useful online resource for epigenetic research. As standalone software, Epidaurus is specifically designed to process user customized datasets with both efficiency and convenience.

INTRODUCTION

Epigenetic mechanisms, including DNA methylation, histone modification and chromatin remodeling, play a critical role in various cell functions and processes. Epigenetic aberrations have been linked to the initiation and propagation of many diseases, and epigenetic dysregulation is currently recognized as one of the hallmarks of cancer (1). Differ-
Combining different epigenetic data types requires two types of data combination methods, implemented in two consecutive steps. The first step, referred to as data aggregation, consists of accumulating the epigenomics information across many loci throughout the genome. Aggregation analysis is a holistic approach that summarizes epigenetic scores from many genomic regions and therefore provides a global view of the epigenomic landscape of these genomic regions. Such an analysis could be applied to genome regions such as TFBSs, histone modification sites, regions sharing a cognate DNA motif, transcription start sites (TSS). For example, genome-wide aggregation analysis on androgen receptor (AR) binding sites reveals the repositioning of nucleosomes from their original (central) positions to two flanking positions (10). Another study using the aggregation analysis approach finds that 20 nucleosomes are well-positioned around CCCTC-binding factor (CTCF) binding sites, highlighting the important role of CTCF in nucleosome positioning (11). These results demonstrate the power and usefulness of genome-wide aggregation analyses. The second step, referred as data integration, consists of integrating aggregated data of different types such as TF-ChIP-seq, histone ChIP-seq, DNA methylation (MeDIP-seq) and DNase-seq. Data integration facilitates the side-by-side comparison of different data types.

Data visualization assists researchers in exploring relevance and differences among datasets, and in generating and validating hypotheses. UCSC genome browser, Ensembl and IGV provide user-friendly interfaces to visualize and compare genomic and epigenomic signals of many different types as vertically piled-up tracks for a single locus (12–14). However, they are not designed to visualize the results of genome-wide aggregation analyses. Therefore, complementary tools are needed to summarize and visualize epigenomic features and enable the identification of novel associations between these features. Spark is a tool designed to fulfill this goal (15). However, its visualization has limited capability to reveal the relevance and differences between datasets (see ‘Results and Discussion’ section).

In this study, we presented Epidaurus, a bioinformatics tool that can simultaneously perform aggregation analysis of thousands of genome regions and integrative analysis for many epigenetic datasets. To demonstrate its usefulness, we used Epidaurus to analyze the epigenome of castration-resistant prostate cancer (CRPC) in Abl cells (16). Use of Epidaurus enabled us to confirm that transcription repressor EZH2 is uniquely expressed in CRPC and validated our hypothesis (17). When applying Epidaurus to another prostate cancer genome dataset in LNCaP cells (17), we revealed a novel regulating mechanism of AR. Specifically, knockdown of the pioneer factor FOXA1 selectively induces AR to bind promoters, thus reprogramming AR to regulate a set of genes including E2F1 that are not normally androgen stimulated (17). We therefore exemplified in this study that Epidaurus can only validate hypotheses, but can also generate novel biological insights, leading to a deeper understanding of the epigenetic landscape.

**MATERIALS AND METHODS**

**Data collection**

Epigenetic data for LNCaP, VCaP, LNCaP-Abl (Abl), MCF7, GM12878, K562, HeLa-S3, A549 and HePG2 cells were assembled from published data deposited into GEO and Sequence Read Archive. Histone ChIP-seq datasets include H3K4me1, H3K4me2, H3K4me3, H3K9me3, H3K27me3, H3K36me3, H3K79me2, H4K20me1 H4K5ac, H3K27ac, H2A.Z, H2AZac and H3K122ac. Transcription factor ChIP-seq datasets include AR, CTCF, FOXA1, MED12, P300, EZH2, SUZ12, NKK3.1, Pol2, CEBPB, ELF1, FOSL2, FOXM1, GABP, GATA3, E2F1, HDAC2, JUND, MAX, NR2F2, ERG1, CMYC, etc. Chromatin accessibility datasets include DNase-seq and FAIRE-seq. DNA methylation datasets include MeDIP-seq and RRBS. Gene expression datasets include RNA-seq, small RNA-seq and GRO-seq. MNase-seq data from hematopoietic stem cells (CD34+ cells) and their differentiated erythroid lineage cells (CD36+ cells), a leukemia cell line (K562) and a lymphoblastoid cell line (GM12878) were collected. We also prepared genome feature datasets including sequence conservation (PfasCon and PhyloP score), GC content and CpG density. The current Epidaurus database contains 233 datasets (Supplementary Tables S1–S9). This number will increase as more data become available.

**Software implementation**

Epidaurus was implemented in Python and C: the source code and documentation are freely available from our website (http://epidaurus.sourceforge.net/). Epidaurus can be invoked from the command line as well as from our online web server (http://bioinformaticstools.mayo.edu:8080/Epidaurus/). When running from the command line, Epidaurus took two files as input: a configuration file specifying the parameters and paths of all BigWig files (18), and a BED file containing genome regions of interest such as TFBS. Epidaurus was configured by four parameters: *HALF_WINDOW_SIZE*, specifying the window size added to both sides of the middle point of regions defined in the BED file (default = 1000 bp), *HEAD_ROWS*, specifying number of rows Epidaurus would take into calculation (default = 2000), *HM_FORMAT*, specifying the output graphic format (pdf, png or tiff, default = pdf) and *DIST_Metric*, specifying metric to measure distance between two datasets (Pearson, Kendall, Spearman, Euclidean, default = Kendall). The conceptual design of Epidaurus is illustrated in Supplementary Figure S1. Briefly, Epidaurus analysis procedure is detailed in the following steps:

(i) For each row in input BED files, Epidaurus built the genomics window by extending *HALF_WINDOW_SIZE* (bp) up- and downstream from the middle point. If *HALF_WINDOW_SIZE* was set to 0, Epidaurus used the original regions provided in BED file without extension: in this case all genomic regions in input BED file must be the same size.
(ii) Using parameters defined in the configuration file, Epidaurus extracted signals from BigWig files. For example, if there were $K$ BigWig files representing $K$ datasets, $n$ (specified by $\text{HEAD\_ROWS}$) rows in the BED file and the $\text{HALF\_WINDOW\_SIZE}$ was set to $w$ (note the total window size will be $2w + 1$). After signal extraction, Epidaurus generated $K$ data matrices with each matrix having $n \times (2w + 1)$ values.

(iii) For each data matrix, Epidaurus calculated the mean of each column resulting $K$ lists with each list having $2w + 1$ values. The $K$ lists represented signal profiles of $K$ datasets.

(iv) Epidaurus then built the matrix $K \times (2w + 1)$. Values in each row were scaled into range (0,1) using:

$$V_i^* = \frac{V_i - V_{\min}}{V_{\max} - V_{\min}}, \; i \in \{0, 1, 2, \ldots, 2k\}$$

(v) Finally, the heatmap and line graph were generated. The order of datasets displayed in the heatmap was determined by the distances to ‘seed’ dataset specified in configuration file. Distance was measured by one of the four metrics: Pearson correlation coefficient, Kendall rank correlation coefficient, Spearman rank correlation coefficient or Euclidean distance. Details are provided in the section below.

Measuring similarity between two epigenetic datasets

The majority of high-throughput sequencing data (such as RNA-seq, ChIP-seq, MNase-seq) could be represented as a set of genomic positions and the associated scores. Regardless of the data type, Epidaurus computed an epigenetic profile for each selected dataset with single nucleotide resolution. When visualizing these profiles using heatmap, we grouped similar profiles together to facilitate comparison and interpretation.

The similarity between two profiles ($X$ and $Y$) was measured by the distance between the corresponding arrays of values ($x \in \{x_0, x_1, x_2, \ldots, x_{2k}\}$ and $y \in \{y_0, y_1, y_2, \ldots, y_{2k}\}$). We used four different metrics to measure such distance. Euclidean distance ($d$), Pearson’s correlation coefficient ($r$), Spearman’s rank correlation coefficient ($\rho$) and Kendall’s rank correlation coefficient ($\tau$) are defined as:

$$d(X, Y) = \sqrt{(x_0 - y_0)^2 + (x_1 - y_1)^2 + \cdots + (x_{2k} - y_{2k})^2}$$

$$r(X, Y) = \frac{\text{cov}(x, y)}{\sigma_x \sigma_y} = \frac{E[(x - \bar{x})(y - \bar{y})]}{\sigma_x \sigma_y}$$

$$\rho(X, Y) = \frac{2k}{\sqrt{\sum_{i=0}^{2k} (x_i - \bar{x})^2 (y_i - \bar{y})^2}}$$

$$\tau(X, Y) = \frac{\# \text{ of concordant pairs} - \# \text{ of discordant pairs}}{\frac{1}{2} \times 2k \times (2k - 1)}$$

Where $\text{cov}$ is the covariance, $\sigma_x$ is the standard deviation of $X$, $\bar{x}$ is the mean of $X$, $\bar{y}$ is the mean of $Y$, and $E$ is the expectation operator. A pair of observations, $(x_i, y_j)$ and $(x_j, y_i)$, were considered concordant if the ranks for both elements agreed (i.e. if both $x_i > x_j$ and $y_i > y_j$ or if both $x_i < x_j$ and $y_i < y_j$), and they were considered as discordant, if $x_i > x_j$ and $y_i < y_j$ or if $x_i < x_j$ and $y_i > y_j$. If $x_i = x_j$ or $y_i = y_j$, the pair was neither concordant nor discordant.

RESULTS AND DISCUSSION

Validate noncanonical transcription activation function of $EZH2$ in castration-resistant prostate cancer cells

$EZH2$ is a well-known transcription repressor that cooperates with other PRC2 components including $SUZ12$, $EED$ and $RBBP4$ (19). However, Xu et al. demonstrated that $EZH2$ switches its transcriptional repressive function in androgen-dependent prostate cancer to a gene activating function in CRPC, using LNCaP cells as a model of androgen-dependent prostate cancer and Abl cells as a model of CRPC (16). In Abl cells, Xu et al. identified two groups of $EZH2$ binding sites based on H3K27me3 enrichment. Ensemble peaks (i.e. $EZH2$ binding peaks with H3K27me3 enrichment) repress gene expression and solo peaks (i.e. $EZH2$ binding peaks lacking H3K27me3 enrichment) activate gene expression. Using Epidaurus, we systematically reanalyzed the epigenome landscapes at both $EZH2$ ensemble and solo peaks.

Upon reanalyses, we found that solo peaks were narrow and sharp and ensemble peaks were wide and flat (Figures 1 and 2a). The sequence conservation profiles of these two
types of peaks further confirmed this observation (Figure 2b). This was presumably due to the fact that physical dimension of EZH2 protein alone was much smaller than the PRC2 complex, which consists of EZH2 and other cofactors. Here we showed that genome-wide aggregation analysis was able to provide new evidence to validate existing findings.

From the perspective of the epigenome, we reaffirmed that EZH2 ensemble peaks were primarily associated with transcription repression and that solo peaks were associated with transcription activation with multiple evidence. First, Pol II and AR binding signals were much higher in solo peaks than those in ensemble peaks (Figure 2c and d). Second, we found that in agreement with its repressive role, EZH2 ensemble peaks were mainly located in closed chromatin (Figure 1a), and consistent with its activating role, EZH2 solo peaks were located in nucleosome-free regions delineated by the decreased signal in the middle of H3K4me1, H3K4me2, H3K4me3 and H3K9me3 peaks (Figures 1b and 2e–h). Third, H3K4me2 and H3K4me3, promoter-specific histone modifications associated with active transcription (20–22), had much higher enrichment in solo peaks than that in ensemble peaks (Figure 2f and g). Similarly, enhancer-specific histone marker H3K4me1 signals were much higher in solo peaks than in ensemble peaks (Figure 2e). Finally, H3K9me3 is well known for its repressive role in transcriptional regulation (23,24). Our data showed that H3K9me3 signals were almost undetectable in solo peaks but were enriched in ensemble peaks (Figure 2h).

The distinct epigenetic landscapes between EZH2’s ensemble and solo binding sites strongly supported the dual role of EZH2 in transcription regulation in prostate cancer. These results also highlighted the usefulness of Epidaurus and the strength of integrative analysis.

**Altered epigenetic landscape of AR binding induced by FOXA1 knockdown**

FOXA1 is a transcription factor involved in embryonic development and establishment of tissue-specific gene expression and acts as a pioneer factor in chromatin remodeling. As a master regulator of AR, FOXA1 has been extensively studied in prostate cancer (10,17,25–29). It was reported that FOXA1 opened the local chromatin to facilitate AR binding (30). Wang et al. defined three groups of AR binding sites after FOXA1 knockdown: lost binding (1881 loci, referred as lost AR program), conserved binding (1234 loci, referred as conserved AR program) and gained binding (10 869 loci, referred as gained AR program) (17).

Using Epidaurus, we reanalyzed the genome and epigenome datasets generated from LNCaP cells for these three groups of AR binding sites. We demonstrated that all three groups of AR binding regions were highly conserved across 100 vertebrate genomes, hypersensitive to DNase I and enriched for AR and P300 ChIP-seq signals, suggesting the reliability of these AR binding sites (Figure 3). Supplementary Figure S2k and l, o and p). From the Epidaurus results, we observed dramatically different epigenome landscapes between the gained and lost AR programs. In the lost AR program, all histone ChIP-seq data consistently delineated a nucleosome free region flanking the center of AR binding sites (Figure 3a). In particular, active enhancer markers H3K4me1, H3K4me2, H3K27ac and histone variant H2A.Z clearly exhibited a symmetrical, bimodal pattern with reduced nucleosome occupancy at the central nucleosome and concomitant increased occupancy.

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**Figure 2.** Comparison of signal intensity between EZH2 ensemble peaks (red curves) and solo peaks (blue curves). (a) EZH2 ChIP-seq signal intensity profile. (b) PhastCon conservation score profile. (c) - (f) ChIP-seq signal intensity profiles for Pol2, AR, H3K4me1, H3K4me2, H3K4me3, H3K9me3 and H3K27me3, respectively. All ChIP-seq data were generated from Abl cell line. EZH2 ensemble (red) and solo (blue) and peaks were defined by Xu et al. (16). In each panel, the x-axis is the distance to peak center (bp) and the y-axis is tag intensity.

**Figure 3.** Distinct epigenome landscapes between ‘lost’, ‘conserved’ and ‘gained’ AR programs. Comparison of epigenome landscapes between lost (a), gained (b) and conserved (c) AR programs. The loss, gained and conserved AR binding sites induced by siFOXA1 were defined by Wang et al. in LNCaP cells (17). For each AR binding site, we extended 1-kb to up- and downstream of the peak center. In the heatmap, each row is a dataset and each column is genomic position. Signals of each dataset were normalized into range (0,1). Magenta and cyan colors indicate high and low signals, respectively. MeDIP-seq, DNaseI-seq, GRO-seq and all ChIP-seq data were generated from LNCaP cells treated with dihydrotestosterone. CpG density was computed from the human reference genome (hg19/GRCh37) and the PhastCon score was downloaded from the UCSC annotation database. All heatmaps were generated by Epidaurus.
at two flanking nucleosomes (Supplementary Figure S2g–j). In contrast, for the gained AR program, most AR binding sites were located to the central, well-positioned nucleosome, as shown by the unimodal signal of H3K4me1, H3K4me2, H3K27ac, H3K36me3, H4K5ac, H2A.Z and H3K9me2 (Figure 3b, Supplementary Figure S2d–j). This distinct nucleosome architecture between gained and lost AR programs highlighted the dynamics of nucleosome and the role of FOXA1 as pioneer factor in chromatin remodeling (27,31,32).

However, signals of enhancer-specific histone markers such as H3K4me1, H3K4me2 and H3K27ac were much higher in the conserved AR program than those in the lost AR program, suggesting the conserved AR binding sites had much higher intrinsic enhancer activity and thus were independent of the pioneer effect of FOXA1 on gene activation (Supplementary Figure S2g, i and j). This assumption was substantiated by the observation that the eRNA abundances as measured by GRO-seq as well as the Pol II ChIP signals were also much higher in the conserved AR program than those in the lost AR program (17,33–35) (Supplementary Figure S2m and n).

Cytosines in CpG dinucleotides can be methylated to 5-methylcytosine, which spontaneously deaminate to form thymidine residues over time. Therefore, the CpG dinucleotide is greatly under-represented in the human genome at only about one-fifth than would be expected (36) (Supplementary Figure S3). Genome regions with a high concentration of CpG sites are known as CGIs (37). About 70% of CGIs are located within 2-kb regions flanking TSS (Supplementary Figure S4). On the other hand, the majority (>85%) of AR binding sites are distal from the TSSs of AR regulated genes (26,27,38). Because of this, the chance of observing overlaps between AR binding sites and CGIs is conceivably very slim. To estimate how many AR binding sites overlapped with CGIs by chance, we shuffled AR binding sites and then overlapped them with CGIs (28 691 regions, total 21 842 742 bp or 0.7% of the human reference genome) downloaded from the UCSC annotation database. We estimated that 0.97 ± 0.09% of the AR binding sites would overlap with CGIs by chance (Supplementary Figure S5). Interestingly, we found that the average CpG density was much higher in gained AR binding sites than that in the lost and conserved AR binding sites (Figure 3, Supplementary Figure S6). This suggested the co-localization of CGIs with AR binding sites in the gained AR program. As shown in Figure 4a, 3.06% (333/10 867) of gained AR binding sites overlapped with CGIs, which is 3.15-fold enrichment (P < 2.2E-16, χ² test) compared to background (0.97%), suggesting gained AR binding sites tended to colocize with CGIs. As a comparison, only 0.05% (1/1860) of lost AR binding sites overlapped with CGIs, a 23.4-fold depletion (P = 1.02E-4, χ² test) compared to the background control, suggesting that lost AR binding sites tended to localize outside of CGIs. The overlap between conserved AR binding sites and CGIs was about 1.0% (13/1297), approximating background control.

We further investigated the genome position of gained AR binding sites overlapped with CGIs. Since the majority of CGIs are located in promoters, as expected, we found, as expected, that these AR binding sites were primarily located in promoter regions (Figure 4b), De novo motif search using MEME-ChIP (39) showed significant enrichment of palindromic AR motifs (E-value = 2.4E–158), suggesting the reliability of these AR binding sites (Supplementary Figure S7). We then investigated the genes targeted by gained AR binding sites that overlapped with CGIs. Disease ontology analysis suggested that the target genes were significantly associated with various types of cancers as well as other transcription factors such as SOX2, ETS1, HNF4α and CREB (Figure 4c and d). Genes such as KLK3, EGRF and GAS2LI play critical roles in prostate cancer pathogenesis and progression. Overexpression of KLK3 was widely used as a marker for early prostate cancer detection (PSA test) for decades until its recent suspension. Activation of EGFR is one of the mechanisms accounting for the maintenance of AR signaling in hormone poor environments (such as CRPC) (40,41), and intense efforts have been focused on the development of therapeutic strategies to block EGFR signaling in prostate cancer (42–47). GAS2LI is an ERG-dependent AR activated gene and frequently silenced in prostate cancers (48–50). We analyzed the expression of these four genes using Illumina human-6 v2.0 beadchip (GSE27682) (17), and found that their expressions were significantly up regulated in siFOXA1 LNCaP cells treated with androgen. The t-test P-values for KLK3, EGRF, SUN2 and GAS2LI were 0.0003, 0.049, 0.01 and 0.044, respec-
Figure 5. Examples of gained AR binding (highlighted in light blue) at promoters after FOXA1 knockdown. Screenshots taken from UCSC genome browser showing 4 genes that had AR binding at their promoters. From top to bottom: KLK3 (a), EGFR (b), SUN2 (c) and GAS2L1 (d). Six tracks are displayed for each panel: AR binding in normal condition (red), AR binding with siFOXA1 (brown), H3K4me3 promoter marker (blue), CpG density in 50-bp window (green), CpG islands defined by UCSC (dark green) and gene model (dark blue). AR, AR (siFOXA1), FOXA1 and H3K4me3 ChIP-seq data were all generated from LNCaP cells treated with dihydrotestosterone.

dihydrotestosterone.

H3K4me3 ChIP-seq data were all generated from LNCaP cells treated with dihydrotestosterone. Binding with siFOXA1 (brown), H3K4me3 promoter marker (blue), CpG density in 50-bp window (green), CpG islands defined by UCSC (dark green) and gene model (dark blue). AR, AR (siFOXA1), FOXA1 and H3K4me3 ChIP-seq data were all generated from LNCaP cells treated with dihydrotestosterone.

Figure 6. ChIP qPCR validations. ChIP (Chromatin immunoprecipitation) qPCR analyses to confirm gained AR binding on promoters of KLK3 (a), EGFR (b), SUN2 (c) and GAS2L1 (d). NT, non-target shRNA; shFOXA1, shRNA knockdown FOXA1.

Confusing that these binding events were independent of FOXA1 (17) (Figure 4f).

Although FOXA1 has been extensively studied, its functions in prostate cancer are controversial and not fully understood. FOXA1 expression levels have been associated with both good and bad clinical outcomes depending on the patient cohort (17,26,28). It was reported that FOXA1 expression is slightly up-regulated in localized prostate cancer because cell proliferation is the main feature in this stage, but is remarkably down-regulated in CRPC because cell motility and epithelial-to-mesenchymal transition are essential at this stage (52). Systematic analysis also suggested that FOXA1 is a key factor in the initiation of lung cancer metastasis (53). Therefore, FOXA1 plays different roles in cancer development and progression. Through integrative analysis of the prostate cancer epigenome using Epidaurus, we revealed a novel mechanism for FOXA1 regulation of AR binding to promoter regions. Specifically, we found that knockdown of FOXA1 increased binding of AR to promoter regions. Consistent with our finding, Sharma et al. also found that a larger proportion of AR binding sites were associated with promoter regions in CRPC than in castration-responsive prostate tumor or cell lines (54). We found that the knockdown of FOXA1 induced AR to bind the promoter of EGFR and up-regulate its expression. Interestingly, EGFR activation is one of the mechanisms to activate AR via phosphorylation in androgen-poor conditions such as CRPC to maintain AR signaling (40,41). However, the exact regulatory mechanisms of this feedback loop remained unclear.

Comparing Epidaurus to the existing platform Spark

When compared to the existing data exploration platform, Spark, Epidaurus produced a more accurate representation for the same datasets using the same list of lost AR bindings (Figure 7). This was most likely due to the ca-

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provides informative visualization of the epigenome. By as-
tras, such as Epidaurus, are such tools that facilitate the holistic analysis and
due to the lack of convenient bioinformatic tools. Epidaurus is a tool that facilitates the holistic analysis and
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ously, the application of Epidaurus is not limited to prostate
cancer epigenome studies. Tremendous epigenetic data for
lead to a deeper understanding of prostate cancer. Obvi-
as exemplified in this study that large-scale integrative
ous, compared to Spark, which normalizes the whole
datasets and genome coordinates. (a) Heatmap generated by Spark. (b) Heatmap generated by Epidaurus.
pability of Epidaurus to normalize each dataset indepen-
dently, compared to Spark, which normalizes the whole
datasets. In practice, it is difficult to render data from dif-
derent datasets and different types of data comparable for
several reasons. First, sequencing depth and DNA fragment
size can be considerably different between datasets, often
with an order of magnitude difference between ChIP-seq
data published years ago and that published recently. Sec-
ond, even though sequencing depths and DNA fragment
size can be normalized onto the same scale, the signals
of a particular locus (or a list of loci) are still not com-
parable between diffuse, broad-peak (e.g. H3K36me3) ChIP-
seq experiments and localized, narrow-peak (e.g. H3K4me3)
ChIP-seq experiments. Third, the total binding sites can be
considerably different between different transcription fac-
tors. Finally, high throughput sequencing-derived epige-
netic datasets and genome features (such as PhastCon con-
servation score, CpG dinucleotide density) cannot be nor-
malized onto the same scale. However, independent nor-
malization has its own drawbacks: for example, color depth
is not comparable between different datasets or between
different heatmaps. To overcome these limitations, Epidaurus
generated a raw data table to facilitate the direct compar-
sion of the absolute values between datasets (such as in Fig-
ure 2).

CONCLUSION

We exemplified in this study that large-scale integrative
analyses of prostate epigenome could validate previous
findings as well as generate novel biological insights and
lead to a deeper understanding of prostate cancer. Obvi-
ously, the application of Epidaurus is not limited to prostate
cancer epigenome studies. Tremendous epigenetic data for
other cancer types have been generated, and the data vol-
ume is growing even faster thanks to the dramatic decrease of
sequencing cost. The interactions between different type
of epigenetic data have not been fully explored partially
due to the lack of convenient bioinformatic tools. Epidau-
rus is such a tool that facilitates the holistic analysis and
provides informative visualization of the epigenome. By as-
sembling epigenetic data from public resources, the Epi-
daaurus web server is a useful centralized data hub for epi-
genetic research projects that use cancer cell lines including
LNCaP, VCaP, LNCaP-Abl (Abl), MCF7, GM12878, K562, HeLa-S3, A549 and HepG2. For most of these cell
lines, ChIP-seq data of extensively used histone markers
(such as H3K4me1, H3K4me2, H3K4me3 and H3K27ac)
and chromatin accessibility data (such as DNase-seq and
FAIRE-seq) were prebuilt into our online database. How-
ever, since epigenome is highly dynamic and tends to be cell-
type-specific, standalone Epidaurus is specifically designed
to process user customized, arbitrary datasets with both ef-
ciciency and convenience.

AVAILABILITY

Source code and comprehensive documentation of Epidau-
rus are available at: http://epidaurus.sourceforge.net/. On-
line web server is available at: http://bioinformaticstools.
mayo.edu:8080/Epidaurus/

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Figure 7. Comparison of the visualization effect of Spark to Epidau-
rus. Comparison of Epidaurus with Spark (15) using the same epigenetic
datasets and genome coordinates. (a) Heatmap generated by Spark. (b) Heatmap generated by Epidaurus.
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