

Nucleosomal Context of Binding Sites Influences Transcription Factor Binding Affinity and Gene Regulation

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Transcription factor (TF) binding to its DNA target site plays an essential role in gene regulation. The location, orientation and spacing of transcription factor binding sites (TFBSs) also affect regulatory function of the TF. However, how nucleosomal context of TFBSs influences TF binding and subsequent gene regulation remains to be elucidated. Using genome-wide nucleosome positioning and TF binding data in budding yeast, we found that binding affinities of TFs to DNA tend to decrease with increasing nucleosome occupancy of the associated binding sites. We further demonstrated that nucleosomal context of binding sites is correlated with gene regulation of the corresponding TF. Nucleosome-depleted TFBSs are linked to high gene activity and low expression noise, whereas nucleosome-covered TFBSs are associated with low gene activity and high expression noise. Moreover, nucleosome-covered TFBSs tend to disrupt coexpression of the corresponding TF target genes. We conclude that nucleosomal context of binding sites influences TF binding affinity, subsequently affecting the regulation of TFs on their target genes. This emphasizes the need to include nucleosomal context of TFBSs in modeling gene regulation.

Key words: gene regulation, nucleosome, transcription factor binding site

Introduction

Proper control of gene expression is essential to the complex function of a living cell. Gene expression is regulated at multiple levels, and one of the most important regulation mechanisms is at the transcriptional level. The transcriptional program is controlled by binding of transcription factors (TFs) to the specific DNA sequences in promoter regions of the genes they regulate. Transcription factor binding sites (TFBSs, we refer to active binding sites that are bound by TFs as TFBSs) are thus fundamental to the regulation of gene expression, as they act as the intrinsic signal receivers of genes. Previous studies have revealed that the location, orientation and spacing of TFBSs (*i.e.*, the contexts of TFBSs) can affect gene expression (1–3).

In addition to the contexts of TFBSs mentioned above, nucleosome occupancy also influences binding of TF to DNA. The nucleosome is the fundamental repeating unit of eukaryotic chromatin. It is composed of a histone octamer around which 147 DNA base pairs are wrapped. DNA wrapped in nucleosomes is less accessible than linker DNA, nucleosome

positioning thus plays a profound role in transcription by controlling access of genomic DNA. In general, TF binding shows a preference for nucleosome-depleted regions (4, 5). Consequently, the level of nucleosome occupancy in promoter is inversely proportional to the corresponding gene transcription rate (6). However, a considerable fraction of TFBSs reside in nucleosomes rather than in linker DNA (7, 8). This leaves open the question of whether different nucleosomal contexts of TFBSs have distinct effects on TF binding. The generation of genome-wide nucleosome positioning (9–11) and TF binding data (12) in budding yeast (*Saccharomyces cerevisiae*) allows us to address this question.

In this study, we investigated how nucleosomal context of TFBSs influences binding affinity of TF to DNA and gene regulation. We found that TF binding affinities tend to be negatively correlated with nucleosome occupancy of the associated binding sites. We further showed that this relationship is mainly due to nucleosomal context of binding sites. We also compared gene features of promoters in which bind-

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ing sites are covered by nucleosomes with features of promoters in which binding sites are depleted of nucleosomes. The latter gene cluster exhibits higher gene activities and lower expression noise than the former cluster. Moreover, we found that nucleosome-covered binding sites disrupt coexpression of the corresponding TF target genes.

Results

High nucleosome occupancy of binding site is associated with low TF binding affinity

To examine the genome-wide influence of nucleosomal context of TFBSs on TF binding affinity, we used two datasets that provide a genome-wide measurement of binding affinities of various TFs (12) and nucleosome occupancy (9) in YPD medium. Harbison *et al* have combined chromatin immunoprecipitation and DNA microarrays (ChIP-chip) to provide a quantitative estimate for the binding affinities of 203 TFs to all promoters *in vivo* (12). They have also identified binding sites for each of 102 TFs. In subsequent analysis, we used the dataset of highly significant ($P \leq 0.001$) binding sites for the 102 TFs and mapped measured TF binding affinities to these TFBSs. Using nucleosome occupancy measured with 4-bp resolution (9), we calculated for each TFBS the nucleosome occupancy. We then asked whether TF binding affinity of a binding site is correlated with its nucleosome occupancy. Indeed, TF binding affinities tend to decrease with increasing nucleosome occupancy of the associated binding sites (Figure 1). We further examined the distribution of TF binding affinities along the nucleosome. TF binding affinities tend to increase with increasing distances from the associated binding sites to their nearest nucleosome centers (Figure 2). Consistent with this, nucleosome-depleted TFBSs (~62% of all TFBSs) have significantly higher binding affinities than nucleosome-covered TFBSs (~38% of all TFBSs, $P < 10^{-86}$, Mann-Whitney U-test) (Figure 3). We asked whether nucleosomes covering TFBSs have characteristic features compared to the other nucleosomes. However, there is no significant difference in histone H3 turnover rate (13) and histone variant H2A.Z occupancy (8) between the two nucleosome classes (data not shown). The details of data process are described in Materials and Methods.

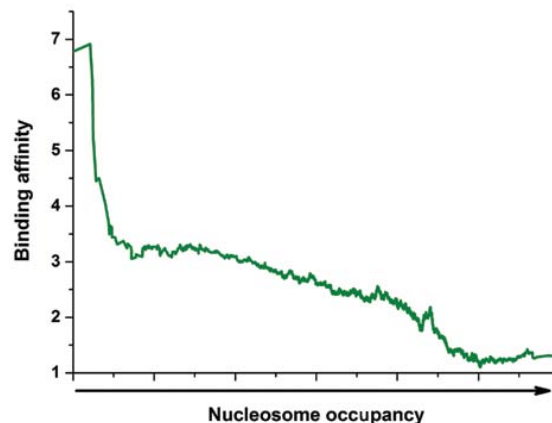


Figure 1 Correlation between nucleosome occupancy of binding site and the associated TF binding affinity. TFBSs were ordered by their nucleosome occupancy, and the associated TF binding affinities were smoothed over a sliding window with size of 500 TFBSs.

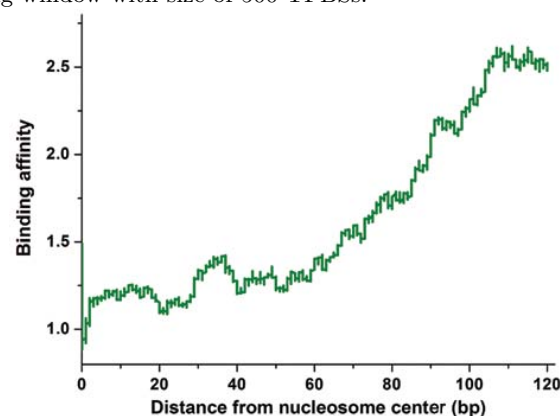


Figure 2 Correlation between the distance of binding site to its nearest nucleosome centers and the corresponding TF binding affinity. TFBSs were ordered by their distances to their nearest nucleosome centers, and the corresponding TF binding affinities were smoothed over a sliding window with size of 500 TFBSs.

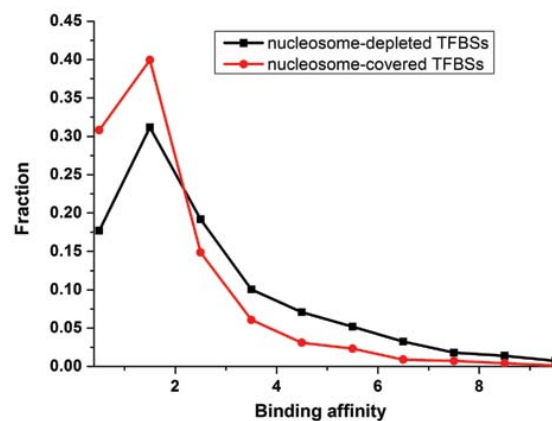


Figure 3 Distribution of TF binding affinities for the two TFBS classes, nucleosome-depleted TFBSs (black) and nucleosome-covered TFBSs (red).

Next, we further examined the relationship between TF binding affinities and nucleosome occupancy of the associated binding sites. TF binding affinity mainly depends on the intrinsic affinity of binding site for the TF and accessibility of binding site, and thus the relationship observed above may be attributable to the intrinsic affinity of binding site for the TF. To test this possibility, we scored each TFBS for a match to the corresponding position weight matrix (PWM) (12). The resulting score is known to provide reasonable approximation for the intrinsic affinity of specific DNA sequence for the TF (14). However, unlike the high correlation between TF binding affinity and nucleosome occupancy (Figure 1), PWM scores are not highly correlated with TF binding affinities (Figure 4). Moreover, PWM scores for nucleosome-depleted TFBSs are comparable to PWM scores for nucleosome-covered TFBSs (Figure 5). These results suggest that nucleosomal context of binding sites could account for most significant difference in TF binding affinities between nucleosome-depleted and nucleosome-covered binding sites.

Nucleosomal context of TFBSs is linked to their distribution at promoter regions

We then tested whether distribution of TFBSs at promoter regions is linked to their nucleosomal context. Indeed, nucleosome-depleted TFBSs show a strong preference for region immediately upstream of transcription start site (TSS), whereas nucleosome-covered TFBSs are distributed more uni-

formly throughout promoters (Figure 6). The peak distribution of nucleosome-depleted TFBSs is consistent with a well-known substantial nucleosome-free region (NFR) directly upstream of the TSS (9). We next sought to understand how such TFBSs are depleted of nucleosomes. It has become evident that the genomic DNA sequence is an important determinant of nucleosome positioning (15, 16) and specific sequences inhibit nucleosome formation (17, 18). In particular, DNA rigidity is generally disfavored by nucleosomes (19). In the opposite manner, we scored each trinucleotide in terms of bendability as defined in a previous study (20). Specifically, promoters only containing nucleosome-depleted TFBSs display lower bendability (*i.e.*, higher rigidity) directly upstream of the TSS than promoters only containing nucleosome-covered TFBSs (Figure 7). This sequence feature might prevent nucleosome formation, thereby facilitating TF binding. A previous study has found that rigid DNA is associated with TATA-less promoters (21). However, we found that enrichments of TATA boxes are comparable between promoters only containing nucleosome-depleted TFBSs and promoters only containing nucleosome-covered TFBSs (data not shown).

We next examined whether other factor is linked to nucleosome-depleted TFBSs. A variety of protein factors besides genomic DNA also contribute to nucleosome positioning (22): TFs could compete with nucleosomes for occupancy along the genome, and chromatin modifiers could regulate nucleosome mobility. We speculated that TFs of which binding sites tend to be depleted of nucleosomes should cooperate with chromatin modifiers for nucleosomal modulation.

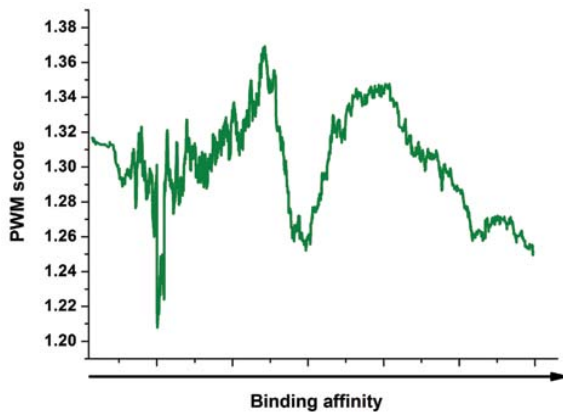


Figure 4 Correlation between PWM score of binding site and the associated TF binding affinity. TFBSs were ordered by their binding affinities, and the corresponding PWM scores were smoothed over a sliding window with size of 500 TFBSs.

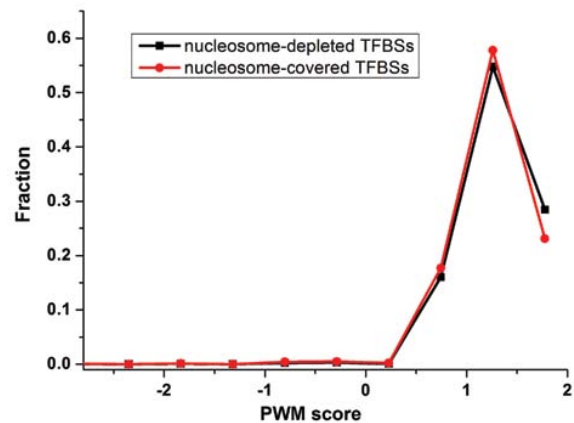


Figure 5 Distribution of PWM scores for the two TFBS classes, nucleosome-depleted TFBSs (black) and nucleosome-covered TFBSs (red).

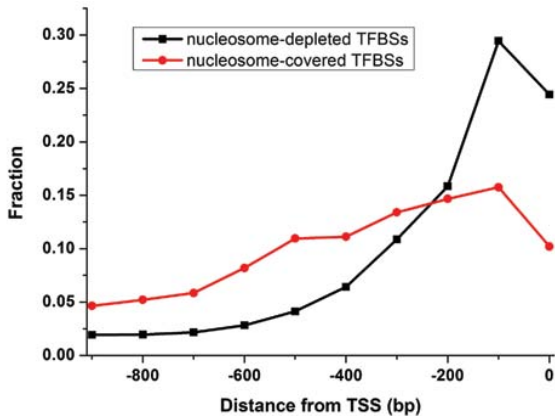


Figure 6 Distribution of TFBSs relative to transcription start site (TSS) for nucleosome-depleted TFBSs (black) and nucleosome-covered TFBSs (red).

The recent genome-wide measurement of occupancy around TSS for seven chromatin modifiers (23) allows us to test this possibility. We identified two promoter classes containing only nucleosome-covered TFBSs and only nucleosome-depleted TFBSs, respectively. Indeed, the seven chromatin modifiers (23) show significantly higher occupancy in nucleosome-depleted TFBS-contained promoters than in nucleosome-covered TFBS-contained promoters ($P < 0.007$, Mann-Whitney U-test). Taken together, the organization of nucleosome-depleted TFBSs could be linked to the underlying DNA sequence and protein factors.

Nucleosomal context of TFBSs is associated with gene regulation

We also examined the relationship between nucleosomal context of TFBSs and gene features. To this end, we first identified three promoter classes associated with only nucleosome-covered TFBSs, only nucleosome-depleted TFBSs, and both nucleosome-covered and nucleosome-depleted TFBSs, respectively. We found that nucleosomal context of TFBSs is correlated with gene activity (24): Nucleosome-depleted TFBSs are associated with high gene activities, whereas nucleosome-covered TFBSs are associated with low gene activities (Figure 8). We further analyzed gene activity in various conditions for the three gene classes. We compiled gene expression data from 1,082 published microarray experiments under various cellular conditions. For each gene, we calculated the proportion of experiments in which it displayed significantly up-regulated expression changes,

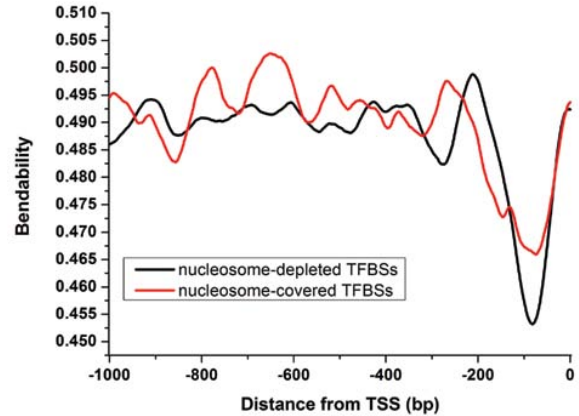


Figure 7 The pattern of DNA bendability relative to TSS for promoters only containing nucleosome-depleted TFBSs (black) and promoters only containing nucleosome-covered TFBSs (red).

and defined the normalized resulting value as open rate. The open rate reflected the general gene activity in various conditions. Genes only containing nucleosome-depleted TFBSs in promoter regions show significantly higher open rates (Figure 8), indicating that the relationship between nucleosomal context of TFBSs and gene activity is conserved among various conditions. Moreover, genes only containing nucleosome-depleted TFBSs in promoter regions show

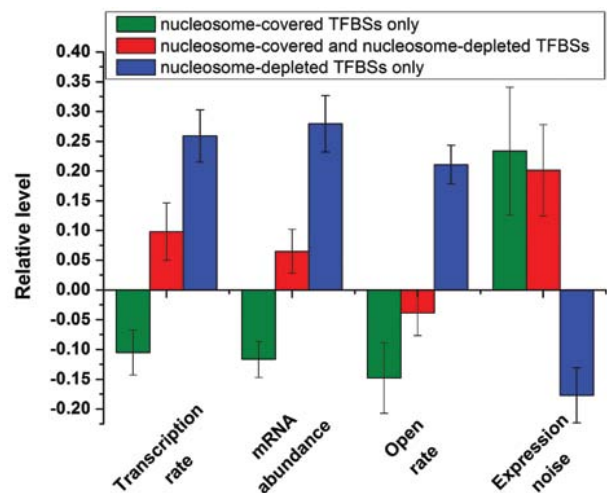


Figure 8 Gene features that distinguish the three promoter classes. Average values that correspond to transcription rate, mRNA abundance, open rate and expression noise are shown for promoters only containing nucleosome-covered TFBSs (green), promoters containing both nucleosome-depleted and nucleosome-covered TFBSs (red), and promoters only containing nucleosome-depleted TFBSs (blue). Values in each property were normalized, such that their means are zero and standard deviations are one. Error bars were calculated by bootstrapping.

significantly lower gene expression noise (Figure 8) (25). Along with the observation above that nucleosome-depleted TFBSs exhibit higher TF binding affinities, these results suggest that stable TF binding is linked to low expression variability.

We next examined whether nucleosomal context of TFBSs is linked to TF regulation. In fact, mere TF binding is not sufficient to guarantee gene regulation, and the context of TFBSs has been shown to affect gene regulation (1). Genes coregulated by a given TF are expected to be coexpressed (26, 27). We wondered whether nucleosomal context of TFBSs influences gene coexpression. We used a combined gene expression dataset in 255 conditions covering environmental stresses (28) and cell cycle (29). For each TF, we calculated the average pairwise Pearson correlation coefficient among expression profiles of its target genes (*i.e.*, the TF cohort). We restricted the analysis to TFs with more than 20 target genes. We calculated the fraction of nucleosome-depleted TFBSs for each of 102 TFs. The fraction values vary considerably with TFs, indicating that TFs differ in nucleosomal context of their binding sites. Indeed, there is a modest positive correlation between the resulting correlation coefficient and fraction of nucleosome-depleted TFBSs for each TF ($r=0.35$, $P<0.005$, Pearson correlation coefficient). Moreover, the overall pairwise Pearson correlation coefficients for expression profiles of each TF cohort become significantly higher ($P<10^{-27}$, Mann-Whitney U-test) when we excluded genes only containing nucleosome-covered TFBSs in promoter regions. Taken together, these results indicate that nucleosomal context of TFBSs plays an important role in gene regulation.

Discussion

It is becoming clear that the context of TFBSs is correlated with TF–DNA interactions and gene regulation (30). The uncovered contexts include the location, orientation and spacing of TFBSs. Furthermore, it has been shown that TFBSs tend to be depleted of nucleosomes (4, 5), but a considerable fraction of TFBSs reside in nucleosomes (7, 8). However, the question of how nucleosomal context of TFBSs influences TF binding and gene regulation is still largely unsolved. In this paper, we found that nucleosome occupancy of binding sites is correlated with the corresponding TF binding affinity and subsequent gene regulation. Specifically, high or low nucleosome occu-

pancy of binding sites corresponds to low or high TF binding affinity, respectively. As nucleosomes limit the access of binding sites to TFs, TFs of which binding sites are in nucleosomes should compete with nucleosomes for binding DNA. Such a competition may result in unstable TF binding, consistent with our finding that high nucleosome occupancy of binding site is associated with low TF binding affinity. On the other hand, binding sites depleted of nucleosomes provide chromatin context for stable binding of TFs to DNA.

We also sought to understand mechanisms of how TFBSs are depleted of nucleosomes. First, the intrinsic DNA sequence provides a concrete framework for positioning nucleosomes. Two recent studies have revealed that linker DNA tends to evolve slower than nucleosomal DNA (31, 32), implying that linker DNA may play a more important role in nucleosome positioning than nucleosomal DNA. Rigid DNA is known to inhibit nucleosome formation and tend to be enriched in linker DNA (19). We found a correspondence between rigid DNA and enrichment of TFBSs directly upstream of TSS at promoters only containing nucleosome-depleted TFBSs. This organization could be linked to the co-evolution constraint on linker DNA and TFBSs. Second, a variety of protein factors are also involved in nucleosome positioning. For example, the ATP-dependent chromatin remodeling complex Isw2 has been shown to override the underlying DNA to reposition nucleosomes (33). We found that nucleosome-depleted TFBSs tend to involve more interactions among TFs and chromatin remodelers. This result indicates that more regulatory proteins are required to ensure proper TF binding.

A key finding of this study is that nucleosomal context of TFBSs is associated with their function in gene regulation. Nucleosome-covered TFBSs are linked to low gene activity and high expression noise. This relationship can be accounted by our observation that nucleosome-covered TFBSs have low TF binding affinities. This unstable TF binding could limit their regulatory function, leading to the relatively low gene activities of their target genes. The unstable TF binding might also indicate actual binding only in a subpopulation of cells, resulting in high expression noise of their target genes. While our manuscript was in preparation, the correspondence between nucleosome-covered TFBSs and high expression noise was also reported (34). Moreover, we found that nucleosomal context of TFBSs also affects coexpression of the corresponding TF target genes. The correspondence be-

tween nucleosome-covered binding sites and low co-expression of the associated TF target genes may be due to limited regulatory function of TFs caused by unstable TF binding.

Taken together, we showed that nucleosomal context of TFBSs influences TF binding affinity; as a consequence, the regulation of TFs on their target genes is also affected. This finding should facilitate the understanding of mechanisms of relationship between nucleosome occupancy and gene regulation. Our results also highlight the importance of considering nucleosomal context of TFBSs for a more accurate description of TF-driven gene regulation.

Materials and Methods

Nucleosome positioning and TF binding data

TF binding data were taken from Harbison *et al* (12), which include the binding affinities of 203 TFs to all promoters in YPD medium. For 102 of the 203 TFs, they identified exact binding sites at promoters, respectively. They also calculated PWM from binding sites for each of 102 TFs. A *P*-value cutoff of 0.001 was used to define the set of genes bound by a particular TF. By applying this strict binding threshold, we ensured a low level of false positives. The dataset includes 9,715 binding sites for 102 TFs. We mapped binding sites to their located promoters (1,000 bp upstream of the gene in this study, the upstream region was truncated if it overlapped with neighboring genes), and then mapped these TFBSs with binding affinities of the associated TFs to the corresponding promoters. We also scored each TFBS for a match to the corresponding PWM. The resulting score indicates the intrinsic affinity of binding site for the TF.

Genome-wide nucleosome occupancy data were measured with 4-bp resolution in YPD medium by Lee *et al* (9). They also identified nucleosome positions in terms of nucleosome occupancy using a computational approach, and determined each nucleosome to be well-positioned or fuzzy. We also mapped histone H3 turnover rate (13) and histone variant H2A.Z occupancy (8) to each nucleosome, and used the average across the region it covers for analysis. We calculated for each TFBS the average nucleosome occupancy across the region it covers. For each TFBS, we also calculated the distance from its center to its nearest nucleosome center. We determined nucleosome-

covered and nucleosome-depleted TFBSs by examining whether or not the binding sites are in nucleosomes.

Gene expression data

The transcription rates and mRNA abundance were taken from Holstege *et al* (24), which were normalized, such that their means are zero and standard deviations are one. Gene expression noise was taken from Newman *et al* (25), which were also normalized, such that their means are zero and standard deviations are one. Gene expression data used for coexpression analysis were measured in environmental stresses (28) and cell cycle (29), a total of 255 conditions.

We compiled available gene expression data from the Stanford Microarray Database (<http://genome-www5.stanford.edu>), a total of 1,082 published microarray experiments for 6,260 genes in various cellular conditions. For each gene, we calculated the proportion of experiments in which it displayed significantly up-regulated expression changes, and defined the normalized resulting value as open rate. To avoid confusion due to experimental noise, we set a relatively strict threshold (2.5-fold) for significantly up-regulated expression changes.

Other data

Yeast genome sequences were downloaded from the *Saccharomyces* Genome Database (<http://www.yeastgenome.org>). We scored each trinucleotide in terms of bendability as defined in a previous study (20). The TSS data were taken from David *et al* (35). The genome-wide occupancy of seven chromatin modifiers (ISW1a, ISW1b, ISW2, SWI/SNF, RSC, INO80, and SWR-C) are derived from Venters and Pugh (23). We calculated the mean occupancy around TSS over the seven chromatin modifiers.

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Authors' contributions

ZD designed the study, implemented the algorithms, and carried out the experiments. ZD and XD analyzed the results and drafted the manuscript. QX and JF participated in the analysis and discussion. All authors read and approved the final manuscript.

Competing interests

The authors have declared that no competing interests exist.

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