Supplemental Information

Transcriptional Regulators

Compete with Nucleosomes Post-replication

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Supplemental Experimental Procedures

Tandem H3-MINCE-ChIP
Cell culture, EdU labeling and chromatin preparation until MNase treatment were performed identically to the MINCE-seq protocol. MNase reactions were stopped by addition of EGTA to a final concentration of 2 mM, and samples were clarified by centrifugation at 16,000 rcf for 3 minutes. The supernatant was incubated with H3 antibody (Abcam 1791) overnight followed by incubation with Protein A dynabeads (Life Technologies) for 1 hour. The unbound fraction was separated and beads were subjected to washes according to a published ChIP protocol (Skene and Henikoff, 2015): 1x TSE1, 4x TSE2, 1x Buffer 3, 1xTE. The wash buffers are as follows: TSE1 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), 150 mM NaCl); TSE2 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), 500 mM NaCl); Buffer 3 (0.25 M LiCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl (pH 8.1)). DNA was isolated from input and H3 pull-down material similar to the MINCE-seq protocol. Biotinylated DNA from the purified DNA (H3_input and H3_pull-down) was separated using M-280 streptavidin dynabeads (Life Technologies). The primers used for qPCR are listed in Table S2.

Sequencing
Cluster generation and 25 rounds of paired-end sequencing were performed by the FHCRC Genomics Shared Resource with an Illumina Hi-Seq 2500. Base-calling, data processing and analysis were performed as described (Henikoff et al., 2011). Paired-end sequencing data were processed and aligned with Novoalign (Novocraft; http://www.novocraft.com) against dmel_r5.51.

Computational analysis
Steady state nucleosome positions were called using DANPOS (Chen et al., 2013) with the fragments of size 120-180 bp after combining the input datasets of newly replicated and 1 hour chase experiments. We defined the +1 nucleosome position as the first nucleosome position downstream from the TSS that falls between TSS+50 and TSS+200. To generate plots showing nucleosome size class, we used fragments in the size range of 134-160 bp. All profiles relative to a feature (TSS, +1 position, enhancer site, etc) were plotted as the number of reads spanning a given position relative to the feature divided by the average number of reads over a ±1000 bp window around the feature midpoint. This calculation of enrichment circumvents biases due to local GC content and copy number variations. Genes active in one cell line and not in other were identified by significant nucleosome occupancy at the promoter compared to the +1 position in the non-expressing cell line.

Table S1 Yeast primers used in spike-in experiment, Related to Figure 1

Primer Set Name	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Product size
P1	ACCCCAGATCTCAAACTACTCTC	ACCATCTTGTCTCGTGGTCC	110
P2	CCCCTCTTGCTATGATTCTCTCT	TTGGGACGATGTATGGGCAT	90

Table S2 *Drosophila* primers used in tandem H3-MINCE-qPCR, Related to Figure 2

Primer Set Name	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Product size
P8 (ORC)	TTGGCGACGACAACAATAAA	GAGAAAGCTCTTCGCAAGCA	101
FBgn0260407	CAAATTACGATTACGACATGCAG	GTTAAGCTCACAGCGGCAAC	90
FBgn0086679	CGCGACGAGTAATTCGTGTA	CCACTTGTTATCGACCACCTG	82
FBgn003688	AAGCAGTGTGACCATCTCTGAA	CCGCAAACGAAAACTAAAGG	81
FBgn0261556	CAAAATAAACTATTCGCAGAACG	CGCTTCAGCTTTTTCAGTTG	82
FBgn0023530	CAGATTCAGAGATGCGCAAA	CCCATAACAGGAATGGGTTA	92
CG16833 (Control +1 Nucleosome)	CCTGCGCTTGGGTAGAGTA	TCGCGATTTGTGACCTTTAC	105

Supplemental References

Chen, K., Xi, Y., Pan, X., Li, Z., Kaestner, K., Tyler, J., Dent, S., He, X., and Li, W. (2013). **Danpos: Dynamic analysis of nucleosome position and occupancy by sequencing**. *Genome Res* 23, 341-351.

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