Supporting Online Material for

A Packing Mechanism for Nucleosome Organization
Reconstituted Across a Eukaryotic Genome

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SUPPLEMENTARY ONLINE MATERIAL
Contains Materials and Methods and Supplementary Figures.

Author contributions
Z.Z. conceived of and executed the bioinformatics analyses, was a main contributor to concept development, and contributed to manuscript writing; C.J.W established and performed the protocol for in vitro genome-wide chromatin reconstitutions, and contributed to manuscript writing; M.W. developed and optimized procedures to isolate native and perturbed chromatin from yeast, including nucleosome library preparation, and conducted analyses on native chromatin; E.W. prepared nucleosome libraries from in vitro reconstituted chromatin for high throughput DNA sequencing; P.K. initiated, designed and supervised in vitro genome-wide chromatin reconstitutions, and co-wrote the manuscript; B.F.P. conceived of the project, supervised its development and integration, and co-wrote the manuscript.

Detailed Methods

In vivo nucleosome preparations

Nucleosomes were prepared as described by Jiang and Pugh (3). Essentially, nucleosomes positions were trapped in vivo by formaldehyde crosslinking. Isolated chromatin was digested with MNase to approximately 80% completion, then H4-immunopurified under stringent conditions that allowed only crosslinked DNA to be retained. Essentially identical results were obtained with H3-immunopurification. The resulting MNase-ChIP DNA was then gel-purified to attain mono-nucleosome sized fragments. Inasmuch as the in vivo pattern and in vitro histone-only pattern are quite dissimilar (Fig. 1A and S5), MNase sequence specificity is not the cause of the in vivo pattern of nucleosome arrays.

Nucleosomal DNA libraries were sequenced using the Illumina GAII instrument. All tag 5' coordinates were shifted by 73 bp in the 3' direction to identify the putative nucleosome dyads. Estimation of consensus nucleosome midpoints are not confounded by heterogeneous fragment sizes because measurements on both the forward and reverse strands serve to define the consensus nucleosome midpoint from opposite directions. These dyad coordinates were then used to create composite and cluster plots.

Native chromatin preparations

A BY4741 strain containing Hht2-TAP was purchased from Open Biosystems. The strain was grown to mid log phase (OD = 0.8). Cells were collected by centrifugation, washed and resuspended in ST Buffer (10 mM Tris-Cl (pH = 7.5), 100 mM NaCl), divided into 125 ml aliquots, and then frozen in liquid nitrogen. Aliquots were thawed and resuspended in 1 ml NP-S Buffer (0.5 mM Spermidine, 0.075% IGEPAL, 50 mM NaCl, 10 mM Tris-Cl (pH = 7.5), 5 mM MgCl2, 1 mM CaCl2). 1 ml bed volume of zirconia beads was added to samples in a 2 ml tube and vigorously vortexed for 2 hr at 4°C. Lysates were collected, spun at 14,000 rpm for 10 min, 4°C, and the supernatant discarded. Chromatin pellets were combined and fully resuspended in NPS Buffer using a dounce. The resuspended chromatin was crosslinked in vitro using 0.6% formaldehyde for 15 min at RT. The reaction was quenched using 125 mM glycine. Chromatin was treated with 30 U Micrococcal Nuclease (Worthington Biochemical, LS004797) for 20 min at 37°C. Digestion was quenched by transferring the samples to ice for 10 min and adding EDTA to 10 mM. The material was spun at 14,000 rpm for 10 min, 4°C, the supernatant was collected and the pellet was resuspended in NP-S buffer with 0.2% SDS and sonicated for 2 cycles of 30 sec each. The material was spun at 14,000 rpm for 15 min, 4°C, the pellet was discarded and the supernatant was combined with the first supernatant.

The supernatant was diluted with NP-S buffer to make the final SDS concentration to
Chromatin immunoprecipitations (ChIP) on MNase-digested chromatin was performed as previously described (5), but with some modifications. 20 µl bed volume IgG Sepharose Fast Flow (Amersham,17-0969-01) was used to capture H3-TAP and was mixed with the MNase-digested chromatin aliquot for 1.5 hrs at 4°C, on a rototorque. The resin was then thoroughly washed with the following series of buffers: once with NPS Buffer, twice with FA Lysis Buffer (50 mM HEPES (pH = 7.5), 150mM NaCl, 2 mM EDTA, 1% Triton X-100, 1% Sodium Deoxycholate), twice with FA Wash 2 Buffer (50 mM HEPES (pH = 7.5), 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 1% Sodium Deoxycholate), twice with FA Wash 3 Buffer (0.25 mM LiCl, 1% IGEPAL, 1% Sodium Deoxycholate, 2 mM EDTA, 10 mM Tris-Cl (pH = 8)), and twice with TE Buffer (10 mM Tris-Cl (pH = 8), 1 mM EDTA). For each wash, 1 ml of the indicated buffer was added, the sample was incubated at RT for 3 min on a rototorque. The resin was gently spun down for 1 min at 2,500 rpm and collected, and the supernatant was discarded. Finally, the resin was resuspended in 450 µl ChIP-Elution Buffer (25 mM Tris-Cl (pH = 7.5), 2 mM EDTA, 200 mM NaCl, 0.5% SDS) and the reaction was allowed to incubate at 65°C for 15 min. The resin was collected by centrifugation at 2,500 rpm for 3 min at RT, and the eluate was transferred to a new tube and incubated with Proteinase K (final amount 30 µg, Roche, 3115828) at 65°C overnight to reverse the crosslinks. DNA was extracted once with phenol:chloroform:isoamyl alcohol and precipitated with 20 µg glycogen and 100% ethanol followed by 70% ethanol wash. ChIP DNA was resuspended in double distilled water with RNase and incubated at 37°C for 2 hours for removing RNA. The samples were sequenced using SOLiD sequencer.

Salt-perturbed chromatin samples were prepared using the same procedure as above but prior to in vitro crosslinking the chromatin was resuspended in NP-S buffer with the indicated salt concentration and incubated at the respective temperature (4°C or 37°C) for 90 min and then crosslinked in vitro.

### Nucleosome reconstitution

**Concept.** The in vitro reconstitution using WCE was based on previous protocols (6-8). Long linear DNA fragments do not assemble well into chromatin and precipitate at rather low histone:DNA ratios. As wrapping of DNA on a nucleosome amounts to about one negative superhelical turn (9), circular and negatively supercoiled plasmids may facilitate a higher degree of nucleosome assembly in SGD compared to linear DNA templates (10). The use of an *E. coli* plasmid library covering the whole yeast genome made SGD more likely to approach the high assembly degrees found in vivo, and avoided the low assembly degrees reported elsewhere (1). Since the prokaryotic portions of the plasmid backbone are poorer templates for assembly (2), they may provide an intra-template buffer to achieve maximal histone occupancy along the *Saccharomyces* portion of the plasmid. Moreover, the use of circular templates avoids effects of DNA ends, which may confound nucleosome positioning preferences especially in the presence of remodeling enzymes (reviewed in Ref. (11)).

**Genomic library preparation.** The library was a gift from Steen Holmberg (University of Copenhagen, Denmark) and originated from a library published elsewhere (12). In short, the library was constructed by insertion of 10-30 kb Sau3A limited digestion fragments of the *S. cerevisiae* genome (strain GRF88, MATa his4-38, S288C background) into vector Ycp50 (ca. 8 kb).

**Whole cell extracts.** Whole cell extracts were prepared as described previously (7) from logarithmic cultures of strain BY4741 (MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0) grown at 30°C in YPD supplemented with 0.1 g/liter adenine and 1 g/liter KH2PO4. Importantly, nucleotides and other small molecules were depleted from whole cell extracts by ammonium sulfate precipitation and dialysis. Moreover, the de novo or salvage pathways present in the whole cell extract are not capable of converting or recycling the added ATP into the three other nucleotides. Thus, assembly reactions were likely not competent for transcription or DNA replication.
**Salt gradient dialysis.** Salt gradient dialysis assembly was carried out as described (7). An assembly mixture contained 10 µg of the genomic library plasmids and sufficient purified *Drosophila* embryo histones (13) to yield a maximum assembly degree as judged by topology assay (fig. S9). This maximum assembly degree corresponded to a similar number of constrained negative supercoils that naturally occur in *E. coli* derived plasmids, which is also indicative of full chromatin assembly. The assembly mixture further contained 20 µg of bovine serum albumin [BSA, A-8022; Sigma] and was in 100 µl high salt buffer (2 M NaCl, 10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 1 mM β-mercaptoethanol, 0.05% IGEPAL CA630 [I-3063; Sigma]). The mixture was dialyzed at room temperature for 15 h while slowly adding a 10 fold volume of low salt buffer (same as high salt buffer but with 50 mM NaCl). Final dialysis versus low salt buffer ensured complete buffer exchange.

**Reconstitution assay.** A reconstitution reaction contained 1 µg of plasmid library DNA pre-assembled into chromatin in a total volume of 100 µl (20 mM HEPES-KOH, pH 7.5, 12% glycerol, 80 mM KCl, 0.5 mM EGTA and 2.5 mM DTT), as described previously (7). Where applicable, whole cell extract (corresponding to about 250 µg protein as measured by Bradford assay with BSA as standard) was added with or without a regenerative energy system (3 mM ATP–MgCl₂, 30 mM (10 mM for non-crosslinked samples) creatine phosphate [Sigma], and 50 ng/µl creatine kinase [Roche Applied Science]). Reconstitution reactions were incubated at 30°C for 2 hours. No additional nucleosome assembly, beyond that occurring in SGD, was detected upon addition of extract (fig. S9), thereby ruling out increases in nucleosome density caused by the extract.

**MNase treatment.** Following the 2 hour incubation, reconstitution reactions were cross-linked by the addition of formaldehyde to a final concentration of 0.05 %, incubated at 30°C for 15 minutes and quenched by the addition of glycine to a final concentration of 125 mM. For the experiments in Fig. 3 crosslinking and immunopurification were omitted. After the MNase treatment, cross-linked reconstitution reactions were incubated with 5 mU (200 mU for non-crosslinked samples) of apyrase [M0393L; New England Biolabs] for 30 minutes at 30°C. Ablation of ATP by 200 mM Apyrase was confirmed using a luciferase assay [Enliten, Promega FF2021]). CaCl₂ was added to a final concentration of 1.5 mM, and samples were digested with variable amounts of Micrococcal Nuclease [N5386; Sigma; note that 1 unit as defined by Sigma corresponds to 85 units as designated for the reconstitution experiments] for 5 minutes at 30°C to give approximately equal degrees of digestion (~20, 250, and 500 units for SGD, WCE-ATP, and WCE+ATP, respectively). Digestion was stopped by adding EDTA to a final concentration of 10 mM.

Samples were brought to 500 µl in NPS buffer and SDS added to 0.05%. Anti-H3 antibodies (10 µg, Abcam) were added and incubated overnight at 4°C. Immunoprecipitations were carried out with 20 µl Magna Protein A beads (1.5 hr 4°C). The immunoprecipitates were washed with FA Lysis buffer +0.025% SDS, then with FA Lysis buffer, then FA High Salt buffer, then FA Wash buffer, then 10 mM Tris-Cl (pH 8), and eluted with 450 µl ChIP Elution Buffer at 65°C for 15 min. Eluted samples were subjected to proteinase K treatment, phenol/chloroform extraction, and ethanol precipitation.

Samples without cross-linking and immunopurification were treated with proteinase K overnight at 55°C directly after MNase digestion, phenol/chloroform extracted and treated with RNaseA, and precipitated with isopropanol. To samples that did not contain WCE 3.5 µl of a 1.5 mg/ml solution *E. coli* tRNA [Sigma] was added as carrier together with proteinase K.

Mononucleosomal-sized DNA fragments were gel purified and subjected to Illumina sequencing. More than 3 million tags were obtained for each sample.

**Topology analysis.** pUC19-PHO8-long plasmids (8) were pre-assembled with histone:DNA mass ratios ranging from 0.5:1 to 1.1:1, added to standard reconstitution assays (with or without WCE and/or ATP) and incubated for 2 hr at 30°C. Samples without ATP contained an additional 1 mM MgCl₂. Afterwards, all samples were treated with 200 mU apyrase [M0393L;
New England Biolabs for 30 minutes at 30°C. Unless indicated otherwise, samples were next incubated with 12.5 U of *E. coli* Topoisomerase I [M0301; New England Biolabs] for 2 hr at 37°C. Reactions were stopped by the addition of 3 µl of 0.5 M EDTA, treated with proteinase K overnight at 55°C, and precipitated with ethanol. Linearized pUC19-PHO8-long was generated by digestion with PstI [New England Biolabs] followed by precipitation with ethanol. Samples were electrophoresed in a 23 cm x 25 cm 1.3% agarose gel in 1X Tris/glycine buffer (384 mM glycine, 50 mM Tris) at 60V (distance between electrodes: 33 cm) for approximately 48 hr. Agarose gel and electrophoresis buffer contained 3 µM chloroquine [C 6628; Sigma]. The gel was stained by shaking in 1X Tris/glycine buffer with 1 µg/ml ethidium bromide for 30 minutes followed by shaking in 1X Tris/glycine buffer without ethidium bromide for another 30 minutes. The DNA was subsequently transferred onto a Nylon membrane [Biodyne B, Pall Filtron] via Southern blotting and hybridized with a radioactively labelled pUC19 vector probe. The radioactive blot was visualized by Phosphorimager (Fuji FLA3000, AIDA software version 4.1.5, PSL modus).

**Bioinformatics**

**Nucleosome mapping.** ELAND (v1.5) and SHRiMP (v1.3.2) were used to map Illumina and SOLiD sequencing tags back to *S. cerevisiae* genome coordinate from www.yeastgenome.org respectively. Only uniquely aligned sequence tags were used for further analysis. After alignment, forward/reverse aligned tags where shifted 73 bp in the 3’ direction to reach their dyad positions. Differences in DNA fragment sizes is accommodated by combining forward and reverse strands, which derives the consensus nucleosome midpoint. Biological replicates of each sample were combined after ascertaining that essentially the same nucleosomal patterns were present in both replicates. Our patterns were similar to published patterns (1, 2) (figs. S2 and S5), thereby excluding methodological differences in data collection and analysis.

**Nucleosome distribution around the TSS.** For every mRNA transcriptional start or end coordinate, defined by the Saccharomyces Genome Database (as of May 13, 2007), the tag distributions of defined regions, 1 kb upstream and 2 kb downstream of the TSS, were retrieved. To eliminate the possibility of biased tag distribution caused by copy number variation in the library preparation, we set the sum of square for each gene’s TSS flanking tag count to 1. Thus each gene contributed equally to clustering and composite landscapes in later steps. Composite landscapes were calculated by aggregating each coordinate relative to the TSS reference point across all genes of a cluster, then dividing by the total gene number in that calculation. In comparing across datasets in composite plots, areas under each curve in the interval -1 kb to +2 kb relative to the TSS were set to be equal (i.e. total tag counts were set to be equal). Tag counts were binned in 5 bp intervals, then subjected to a 9 bin moving average. Our in vivo and in vitro patterns were similar to published in vivo and in vitro patterns (figs. S2 and S5), thereby excluding methodological differences in data collection and analysis.

**Cluster analysis.** Tag distribution from -500 to 1000 bp around the TSS of ~5000 genes with known transcription start sites (TSSs) were clustered by K-means using Cluster 3.0 (14) Mac OSX version on various Ks (from 2 to 10). After comparing cluster result using Java TreeView v1.1.4r3, we chose 5 clusters, which showed distinguished patterns among clusters and least variation within each cluster. The conclusions herein were independent of the value of K. The other datasets were arranged based on the same gene order.

**Nucleosome array plots.** In vivo nucleosome positions were predicted using GeneTrack with exclusion zone of 147 bp and sigma of 20 bp (15). Then the first nucleosome after each TSS was selected as the start nucleosome and the last nucleosome before TES (transcript end site) was selected as the terminal nucleosome. The nucleosome array length was defined as the distance between dyads of the start and terminal nucleosomes. Nucleosome arrays were plotted using Python matplotlib, in which each row corresponds to an array. Arrays were sorted
by array length, and aligned by the array midpoint. For the nucleosome spacing plot (Fig. 4A), a track “feature” within each array (line or row) starts at the one nucleosome dyad and ends at the next adjacent nucleosome dyad, and therefore its length represents internucleosomal spacing. Tracks having spacing lengths of 147 bp to 300 bp were heatmap color-coded from blue (147 bp) to red (300 bp). Tracks >300 bp were color coded white. No track is <147 bp due to nucleosome exclusion defined by GeneTrack. For the nucleosome fuzziness plot (Fig. 4B), fuzziness was measured as the standard deviation of tag coordinates located within the exclusion zone of a nucleosome (16). A track “feature” within each array starts at one fuzziness standard deviation to the left of a nucleosome dyad coordinate and ends one standard deviation to the right. Track features are heatmap color-coded from blue (low fuzziness) to red (high fuzziness). Track lengths and heatmap colors represent redundant information in both Fig. 4A and B. Internucleosome spacing and fuzziness were mapped to 4000 bp around the nucleosome array midpoint.

Fig. 4A quantification of median spacing and standard deviation from the +1 nucleosome to the terminal nucleosome was conducted on all arrays having at least 7 nucleosomes. Plots were smoothed using a moving average of 3 adjacent values.

**Nucleosome turnover rate.** We used lambda_Z column from supplementary Table 2 in Reference (17). Turnover rates for each probe were converted to per bp values, and then were mapped to the TSS. The turnover rates at each coordinate relative to TSS in a particular cluster were summed. In this summation process, the coverage at each base pair relative to each TSS in each cluster was also recorded. Then the summed turnover rate was divided by the coverage to derive the normalized turnover rate on a per base pair basis for each cluster.

![Diagram](image)

**fig. S1.** Illustration that distinguishes nucleosome occupancy level and positioning. Spheres represent nucleosomes resting on DNA “lines”. Occupancy is high when all lines contain a sphere. Positioning is high when all spheres that are present are aligned vertically.
**fig. S2.** Plot of in vivo nucleosome organization used in this study and of the in vivo data of Kaplan et al. (1) showing that the two studies produce nearly identical nucleosomal patterns. (A) Nucleosomal arrays were sorted by array length. (B) Data were aggregated based upon clusters shown in Fig. 1B.
fig. S3. Nucleosome turnover rate for clusters 1-5 (defined in Fig. 1), as a function of distance from the TSS. Turnover rates, as normalized Z-scores are from Dion et al. (17), represent average values aggregated into 10 bp bins. The gray fill backdrop represents the nucleosome distribution of cluster 1.

Clusters 1 and 5 have characteristics of SAGA-regulated/TATA-containing genes (18, 19), in which nucleosomes encroach on the promoter region and/or were intrinsically unstable. Clusters 3 and 4 tended to be TFIID-regulated/TATA-less. Cluster 2 had a strong -1 nucleosome.
fig. S4. Cluster plots of nucleosome organization around nearly all (N = 4785) genes, aligned by their TSS. Uncrosslinked chromatin was isolated from yeast cells and incubated at the indicated NaCl concentration at the indicated temperature for 90 min., then was fixed with formaldehyde. The resulting Native patterns were similar to each other regardless of incubation conditions and also similar to the in vivo pattern obtained after crosslinking in intact cells. Nonetheless, Native patterns were “fuzzier” (std dev. of tags in each nucleosome peak) compared to in vivo (σ = 32, vs 29 for in vivo). The upper left boxed panel is the reference state. YCP50 (lower left panel) is the sequenced genomic library used in Figs. 2 and 3.
fig. S5. Intrinsic nucleosome organization (positioning and occupancy) at the 5’ ends of genes is highly position- and gene-selective. The left set of five panels shows composite traces for the five clusters of genes defined in Fig. 1. The Native pattern is shown as a gray fill. The cluster data are displayed in the lower left panels. “Kaplan et al.” and “Zhang et al.” in vitro data were from their respective studies (1, 2). “SGD” denotes salt gradient dialysis performed on supercoiled plasmid libraries. “600 mM” denotes native chromatin samples that were incubated in 600 mM NaCl at 37°C for 90 min prior to crosslinking. This treatment did not deplete histones from the DNA (see immunoblot in upper right panel). The immunoblot is of H3 and H2B from native (uncrosslinked) chromatin pellets (P) and supernate (S), after treatment with the indicated concentration of NaCl for 90 min at 37°C. The minor band just below 31 KDa is <5% of the signal in all samples, and represents a small amount of H3 tail clippage that has been described by others (4). The 150 and 600 mM samples have slightly more sample loaded, which makes this breakdown product more evident.
fig. S6. Distribution of poly (dA:dT) tracts (red trace) for clusters 1-5 (from top to bottom). Poly (dA:dT) tracts were defined as at least 6 continuous As or continuous Ts but not a mix of them. We searched for at least 6 As or Ts, then our greedy algorithm extended that site to the adjacent nucleotide if it was an A or T. After extending, this site was masked to prevent redundant reporting. Poly (dA:dT) tract mapping to TSS was done using same procedure as was done for nucleosomal tag mapping, except that each nucleotide that was contained in a poly (dA:dT) track was mapped. The Native nucleosome pattern is shown as a gray fill. The graph on the right shows a correlation of poly (dA:dT) tract location and the position of the +1 nucleosome. Each point is a composite distance corresponding to the peak location for each cluster shown in the panels on the left. The +1 nucleosome was defined as described (3).
fig. S7. Cluster plot of nucleosomes reconstituted on genomic DNA by salt gradient dialysis (SGD) at the indicated histone:DNA ratios, then subsequently incubated at 55°C for 1 h 45 min followed by 15 min at 30°C and MNase digestion without cross-linking. For additional reading on the concept of thermal sliding see (20, 21).
**fig. S8.** Positions of the TATA box in the 5' NFR do not correlate with positions of the +1 nucleosome. (A) "+1" nucleosome – TSS distances are plotted versus TATA box – TSS distances (18). (B) Same as panel A, except that peak locations of Reb1 binding sites (n= 476) were used (22). TATA box sites were identified as previously described (18, 22). For Reb1 binding sites, we search the yeast genome for Reb1 motif – TTACCCG. The midpoint of each site was recorded for further calculation.

**Discussion.** If the transcription initiation complex acts as a barrier against which nucleosomes are positioned, the positions of the two should correlate. While these findings do not support a model of global nucleosome positioning by the transcription initiation complex, they do not exclude targeted nucleosome reorganization upon gene activation or repression for which there is ample evidence (23, 24).

The sequence-specific factor Reb1 has been implicated in nucleosome exclusion but not positioning (25-27), and is not part of the pre-initiation complex. The positions of Reb1 sites did correlate with the location of the +1 nucleosome, indicating that Reb1 may play a role in positioning the +1 nucleosome at certain genes.
fig. S9. Incubation with WCE does not increase the histone density on plasmids assembled by SGD. (A) pUC19-PHO8-long plasmid (~6.2kb) was used in a topology analysis as a proxy for the Ycp50 library, which consists of plasmids that are too large and too heterogeneous for this assay. The amount of pUC19-PHO8-long plasmid DNA was the same as that of the Ycp50 library DNA used otherwise. Shown is an ethidium bromide stained agarose gel of pUC19-PHO8-long plasmids assembled with varying histone:DNA ratios and incubated with or without WCE and/or ATP as indicated on top of the lanes. Samples were subsequently treated with topoisomerase I as indicated to remove any supercoils not constrained by nucleosomes, and elec-
trophoresed in the presence of 3 µM chloroquine to reduce the number of negative supercoils such that the topoisomer distribution could be resolved. Without WCE, increasing histone:DNA mass ratios led to a shift of the supercoil distribution downward in the lane confirming the assembly of increasing numbers of nucleosomes on the plasmid templates. The assembly degree asymptotically reached its maximum at the histone:DNA ratio in lane 6 from the left as there is no further shift at even higher ratios. This is apparent even more clearly by the migration positions of the band probably corresponding to the supercoil band of the plasmid dimer. As the maximum assembly degree corresponds to a histone:DNA mass ratio of about 1:1, we define the assembly degree in lane 6 as 1.0 and use it as reference point. As incubation of the assembled plasmids with WCE (with or without ATP) did not shift the band distribution downward, the WCE did not increase the number of nucleosomes on the plasmid templates, neither for the 1.0 nor the 0.5 histone:DNA mass ratio. The presence of ATP caused a slight upward shift in the band distribution, similarly both for the 0.5:1 and 1:1 sample, therefore the relative ratio of assembly degrees was maintained. Arrows indicate the position of the linear and nicked monomeric and dimeric templates, respectively. A majority of templates were nicked, some also linearized, prior to loading due to sheering forces during handling chromatin in vitro (see for example (28)). The lane on the very right contains pUC19-PHO8-long plasmid linearized via digestion with PstI. (B) Same as panel (A), but visualized after Southern blotting and hybridization with pUC19 specific probe.
**fig. S10.** Model for how nucleosomes become organized at the 5’ ends of genes. ATP-dependent chromatin remodeling at the 5’ ends of genes is envisioned to pack genic nucleosomes against a 5’ barrier at canonical distances, in a 5’-3’ graded response. The remodeler(s) may also have or utilize a molecular “ruler” to set linker length. The nature of the barrier is envisioned to be some combination of sequence effects that intrinsically exclude nucleosomes from the NFR and position the first or second nucleosome and/or bind trans-factors that position the nucleosome.
Supplementary References