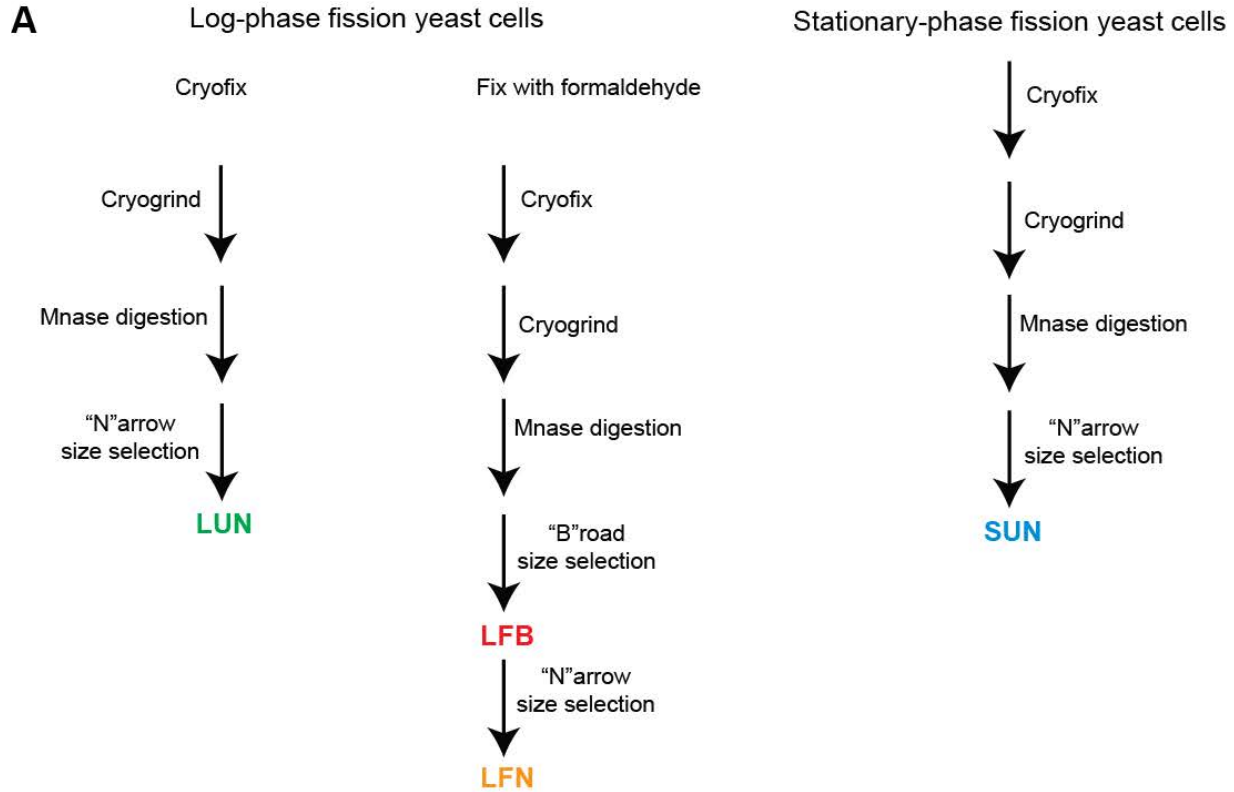
Supplemental Methods

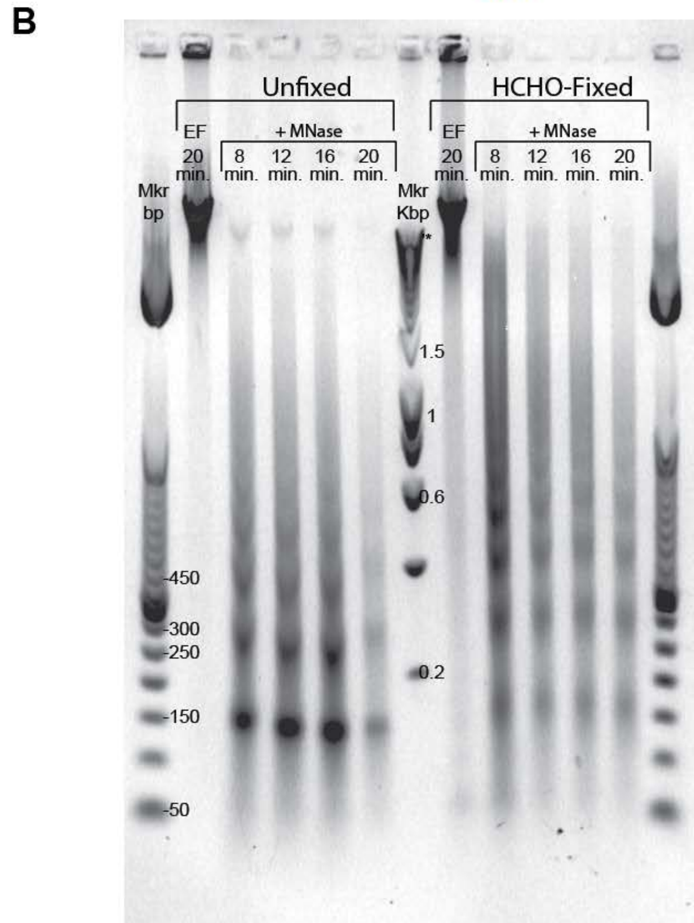
Alignment of origins by eye

We used Igor Pro version 6 (http://www.wavemetrics.com/) to create graphs showing the kinds of data displayed in Figure 5 for all three fission yeast chromosomes. These graphs are available from http://www.acsu.buffalo.edu/~mjbuck/Fission_Yeast_chromatin.html and can be visualized using the free trial version of Igor Pro (from above web site). We developed an algorithm within Igor Pro (available on request from JAH) to generate a series of graphs in PDF format showing selected data from stretches of any desired size of chromosomal DNA centered on each of the positions within a list of chromosomal nucleotide positions. Then we used this algorithm, employing the list called "AT island position" in Table S2, to generate 217 PDF graphs (one for each studied origin) showing stretches of 4 kbp centered on each AT island position.

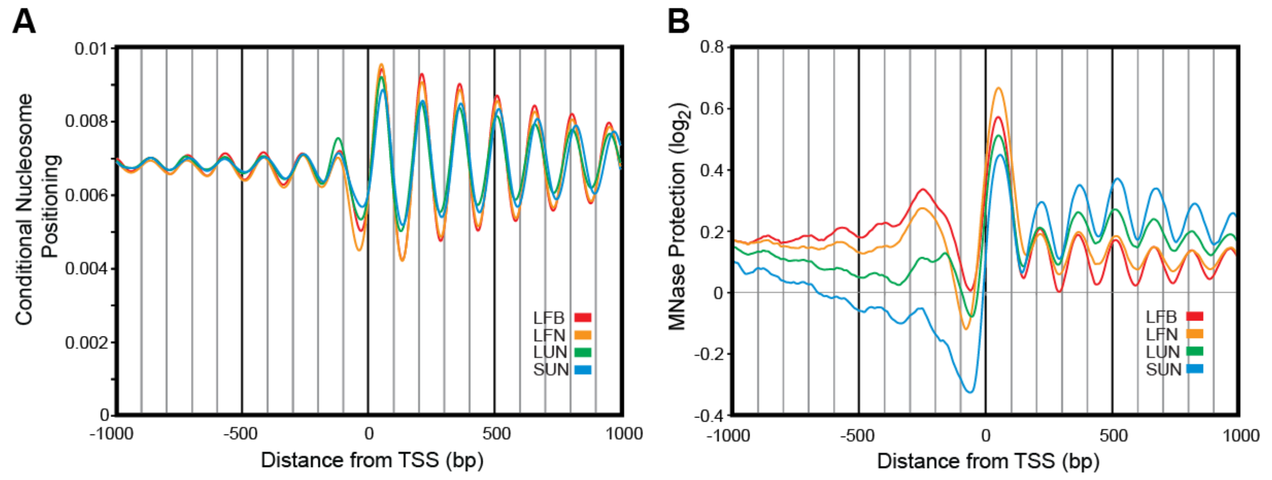
We then scanned these graphs by eye to define *origin centers*. Based on previous observations that yeast replication origins are associated with NDRs (1,2) we used the position of lowest probability of nucleosome formation (black curves in Figure 5, panels ii; (3)) within ±500 bp of the AT island position as our primary criterion for visually-defined origin centers (vdOCs). In cases of ambiguity, we selected the low-nucleosome-probability position with the highest ChIP signal from pre-RC proteins (4). If ambiguity persisted, we added low signal values for all four samples (LFB, LFN, LUN and SUN) as a criterion, ollowed by, if needed, the highest ratio of LFB signal to SUN signal

Finally, we determined origin *orientation* primarily based on whether the bulk of the ChIP signals from pre-RC proteins (4) were to the left ("L") or right ("R") of the vdOC. For a second criterion in cases of ambiguity, we tried to distinguish whether the bulk of the LFB signal was left or right of the vdOC. Note that "R" means the origin was judged to be in the chromosomal orientation (left to right along the standard sequence), while "L" implies the anti-chromosomal orientation.

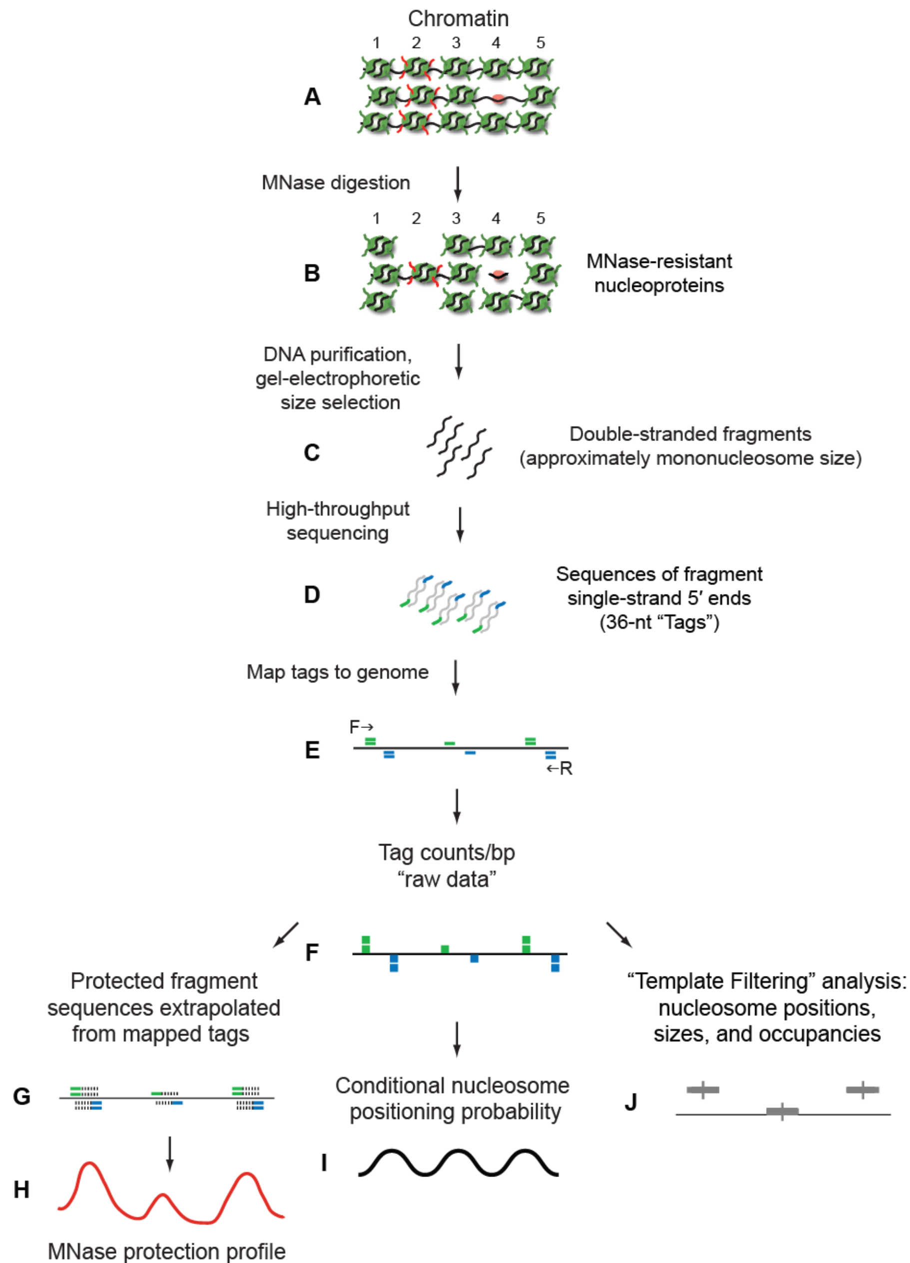




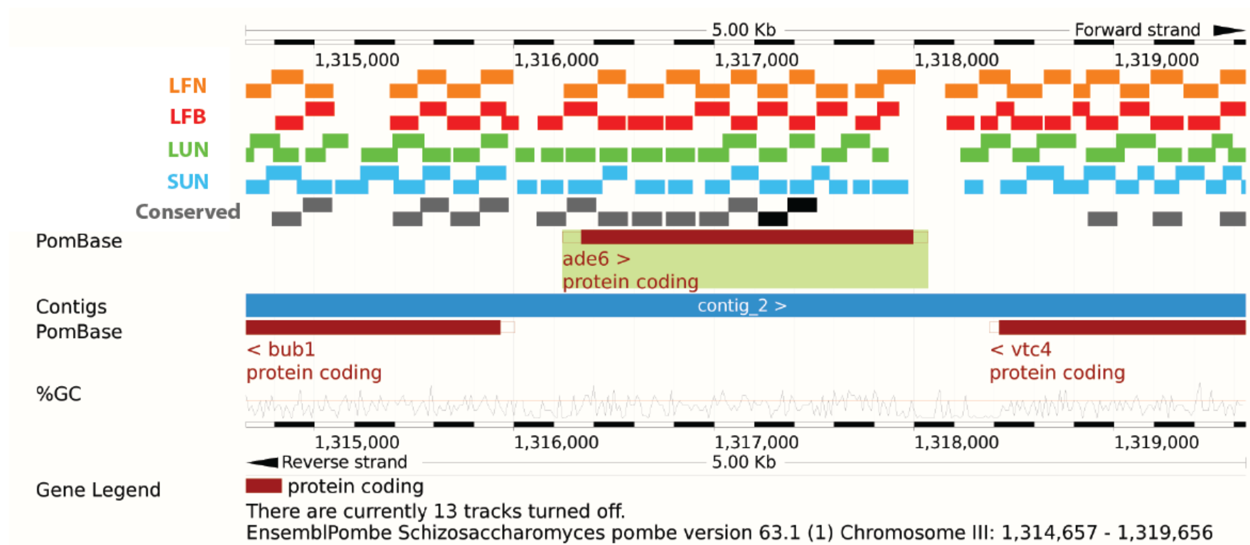
Supplementary Figure S1. (A) Pedigrees of experimental samples. The samples were named according to the following conventions. "**L**" indicates that the cells were growing **L**ogarithmically when harvested. "**S**" means that the cells were in **S**tationary phase when harvested. "**F**" means that the cells were **F**ixed with formaldehyde prior to harvesting, while "**U**" indicates that the cells were **U**nfixed when harvested. "**B**" indicates that the band excised from the prep gel was **B**roader than usual and thus contained a wider range of fragment sizes, while "**N**" means the excised band was relatively **N**arrow, with the intention of analyzing primarily fragments close to mono-nucleosome size. The cells used in the LUN, LFB and LFN samples came from the same batch of log-phase cells, while the cells used in the SUN sample came from an independent stationary-phase culture. For the LUN and SUN samples, the cells were not fixed with formaldehyde. **(B)** Gel-electrophoretic analysis of DNA recovered from MNase-digested nuclei of native ("Unfixed") and formaldehyde-treated ("HCHO-Fixed") stationary phase cells. "EF" indicates exogenous-Enzyme-Free control incubations. "+MNase" denotes incubation at 25°C with 150 units of MNase per ml for the indicated times. "Mkr bp" is a commercial 50-bp DNA-size-marker ladder. "Mkr Kbp" is a commercial 0.2- to 10-kbp DNA-size-marker ladder.



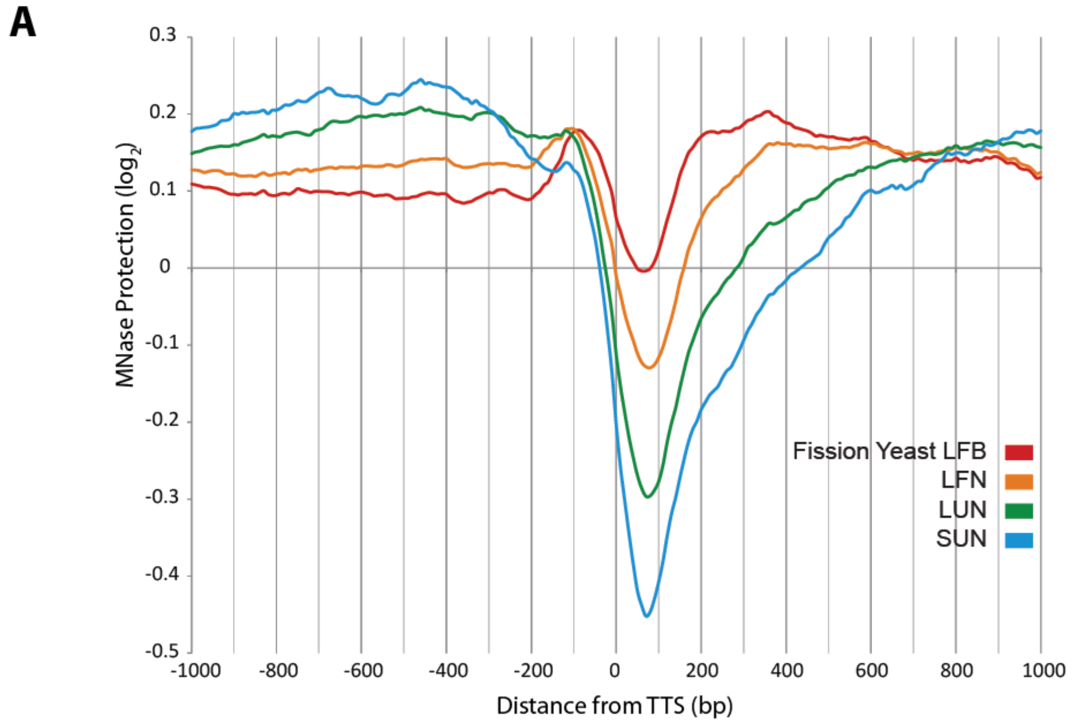
Supplementary Figure S2. (A) Conditional probability of nucleosome dyad at TSSs for all four of our samples over 2001-bp windows centered on transcriptional start sites (TSSs). **(B)** Profiles of MNase protection at TSSs for all four of our samples.

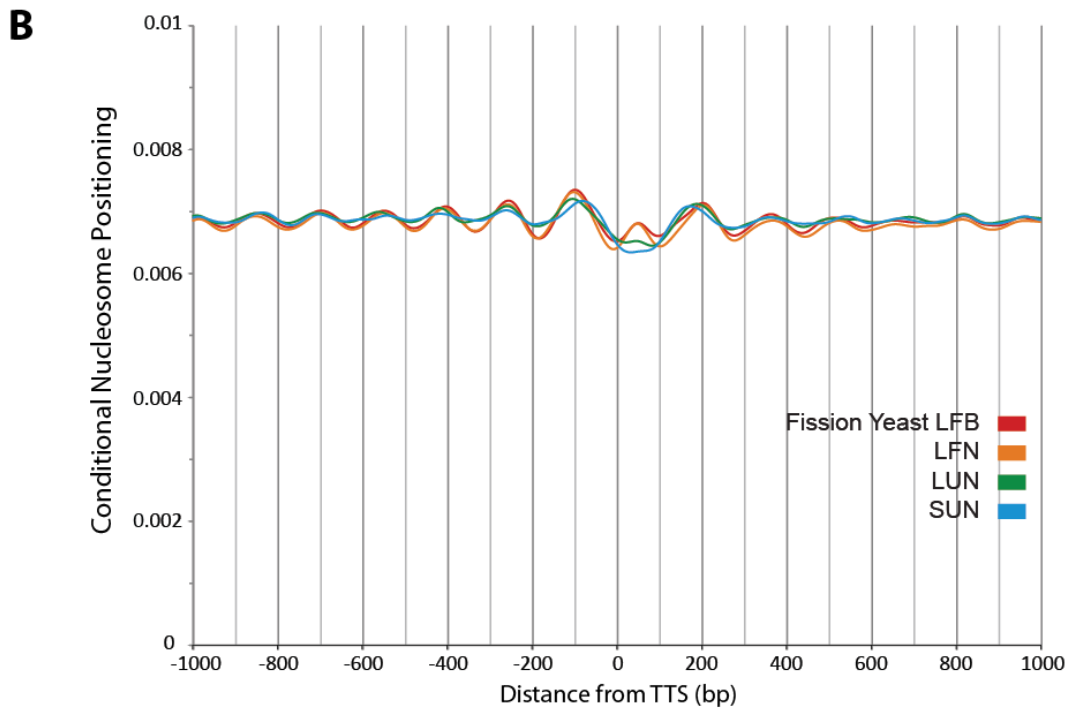


Supplementary Figure S3. Experimental design and data flow. (**A**) Chromatin structure may differ among cells in a population. Black line, DNA. Green ellipses, nucleosomes. Green and red lines, histone tails with differing modifications. Pink ellipse, a non-nucleosomal, DNA-binding protein complex. (**B**) Result of moderate digestion by Micrococcal Nuclease (MNase). (**C**) Double-stranded DNA fragments remaining after gel-electrophoretic selection of mono-nucleosome-sized DNA. (**D**) High-throughput sequencing (Illumina) yields 36 nucleotides of sequence information (a sequence "tag") from the 5′ end of the upper (green) and lower (blue) strand of each DNA fragment from panel **C**. (**E**) The sequence tags are mapped to the fission yeast genome. (**F**) The number of tags that start at each nucleotide position is counted. (**G**) Each tag is computer-extrapolated to a final length of 120 nt. (**H**) The number of times each nucleotide position is contained within a 120-bp extrapolated tag, regardless of strand, is counted. The number of counts at each position is then divided by the genome average for number of counts per position. The resulting numbers are plotted against nucleotide position to generate a "relative MNase-protection profile". (**I**) The raw data in panel F can also be used to calculate "conditional nucleosome positioning probability" (5). (**J**) As an alternative method for viewing nucleosome positions, sizes and occupancies, the "raw data" (tag counts in each strand at each position) from panel **F** are processed by the "template-filtering" algorithm of Weiner et al. (6). The results are represented graphically as horizontal bars centered on the position determined by the algorithm to be the center of that particular nucleosome (short vertical line). The width of each bar shows the algorithm-calculated size of each nucleosome, and the height of each bar is proportional to the average of the separately-calculated occupancies for the upper and lower strands of each nucleosome.

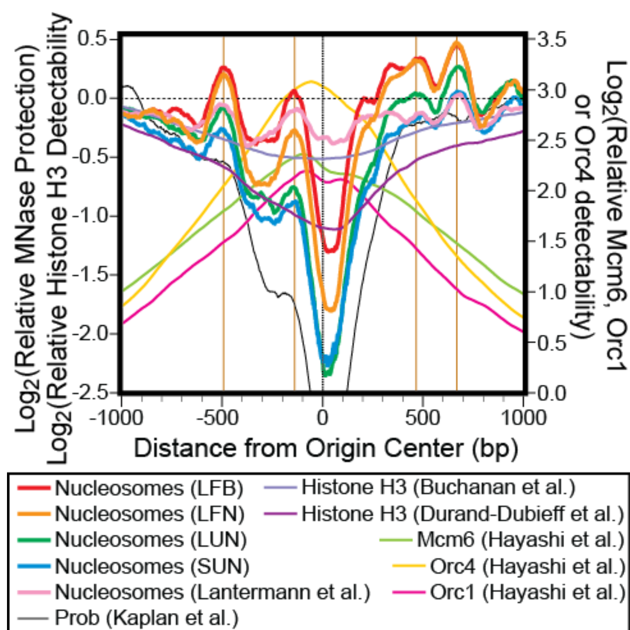


Supplementary Figure S4. Nucleosomes called by template filtering (6) are graphically shown using the Ensembl interface hosted by PomBase (http://www.pombase.org/). Log fixed narrow (LFN - orange), Log fixed broad (LFB – red), Log unfixed narrow (LUN – green), and Stationary unfixed narrow (SUN – blue) are shown in a 5kb window around the ADE6 gene. Conserved nucleosomes are displayed as lenient (grey) and stringent (black).

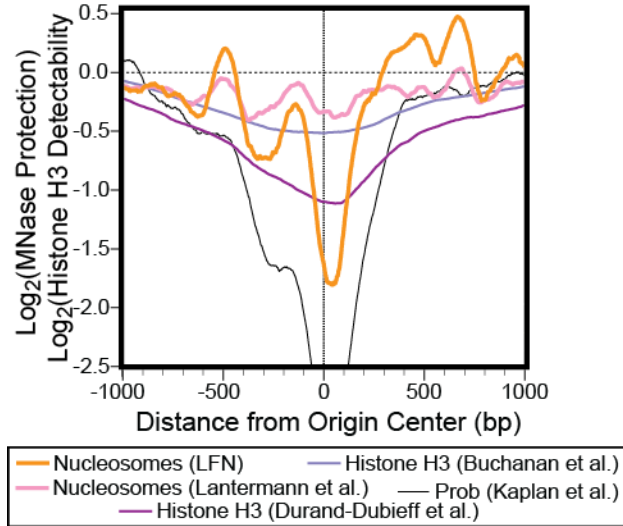




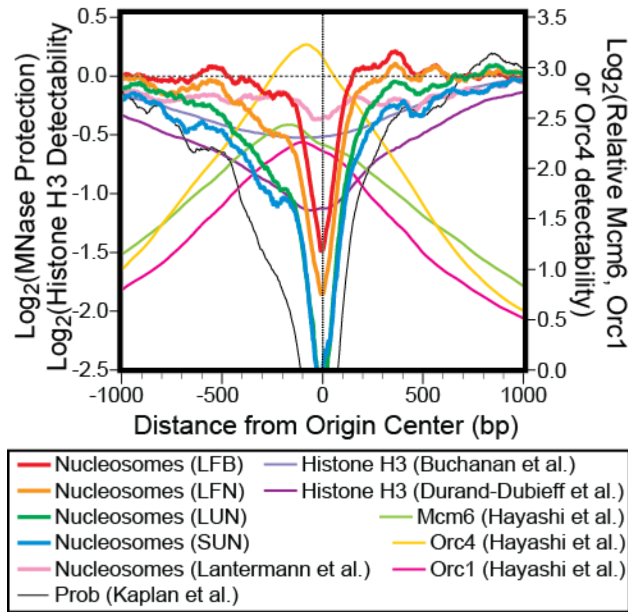
Supplementary Figure S5. (A) Profiles of MNase protection over 2001-bp windows centered on transcriptional termination sites (TTSs) for our four samples. **(B)** Conditional probability of nucleosome start at TTSs.



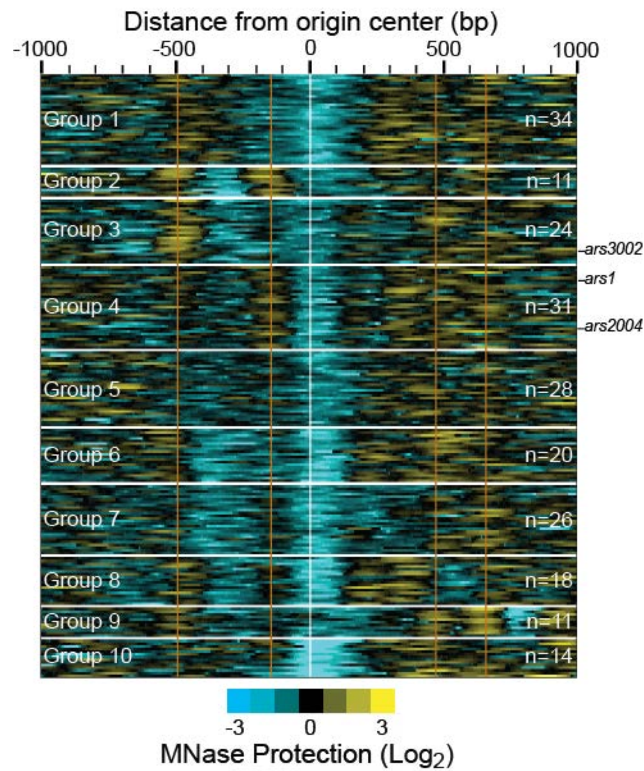
Supplementary Figure S6. Average distribution profiles of MNase protection, histone H3, and pre-RC proteins around 217 replication origins aligned by ArchAlign software. Average MNase protection around replication origins after alignment with ArchAlign, LFN sample (orange line ▮); LFB sample (red line ▮); LUN sample (green line ▮); SUN sample (blue line ▮); data of Lantermann et al. (2) (pink line ▮). Black line, ▮: predicted nucleosome occupancy, based on algorithm of Kaplan et al. (3). Average signal ratios for histone H3. Data from Buchanan et al. ((7); blue-gray line, ▮) and Durand-Dubief et al. ((8); purple line, ▮). Average ChIP-chip signal ratios (log_2) for ORC1 (magenta line, ▮), ORC4 (yellow line, ▮), and MCM6 (yellow-green crosses, ▮). Data from Hayashi et al. (4).



Supplementary Figure S7. Comparison of our MNase-seq results to MNase microarray results. Average MNase protection around replication origins after alignment with ArchAlign for our LFN sample (orange line ▮) and for data of Lantermann et al. (2) (pink line ▮). Black line, ▮: predicted nucleosome occupancy, based on algorithm of Kaplan et al. (3). Average signal ratios for histone H3. Data from Buchanan et al. ((7); blue-gray line, ▮) and Durand-Dubief et al. ((8); purple line, ▮).



Supplementary Figure S8. Average distribution profiles of MNase protection, histone H3, and pre-RC proteins around 217 replication origins aligned by eye. Average MNase protection around replication origins after alignment with ArchAlign. LFN sample (orange line ▮); LFB sample (red line ▮); LUN sample (green line ▮); SUN sample (blue line ▮); data of Lantermann et al. (2) (pink line ▮). Black line, ▮: predicted nucleosome occupancy, based on algorithm of Kaplan et al. (3). Average signal ratios for histone H3. Data from Buchanan et al. (7); blue-gray line, ▮) and Durand-Dubief et al. ((8); purple line, ▮). Average ChIP-chip signal ratios (log_2) for ORC1 (magenta line, ▮), ORC4 (yellow line, ▮), and MCM6 (yellow-green crosses, ▮). Data from Hayashi et al. (4).



Supplementary Figure S9. Groups of origins with similar chromatin structure. K-medioids clustering with k=10 emphasizes heterogeneity among origins. Heat map showing relative MNase protection (LFN sample; color scale at bottom of heat map) for 1000 bp on each side of 217 origins. The heat-map lines corresponding to the well-characterized origins, *ars1, ars2004,* and *ars3002* are indicated.

References for Supplementary Figures

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