SUPPLEMENTAL MATERIAL

The Prenucleosome, a Stable Conformational Isomer of the Nucleosome

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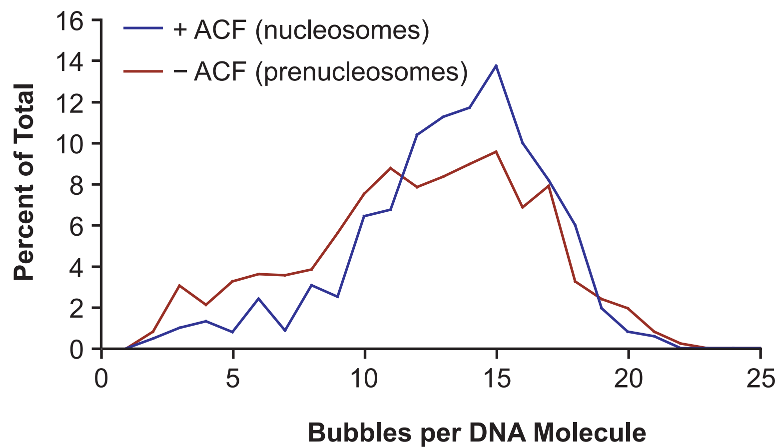
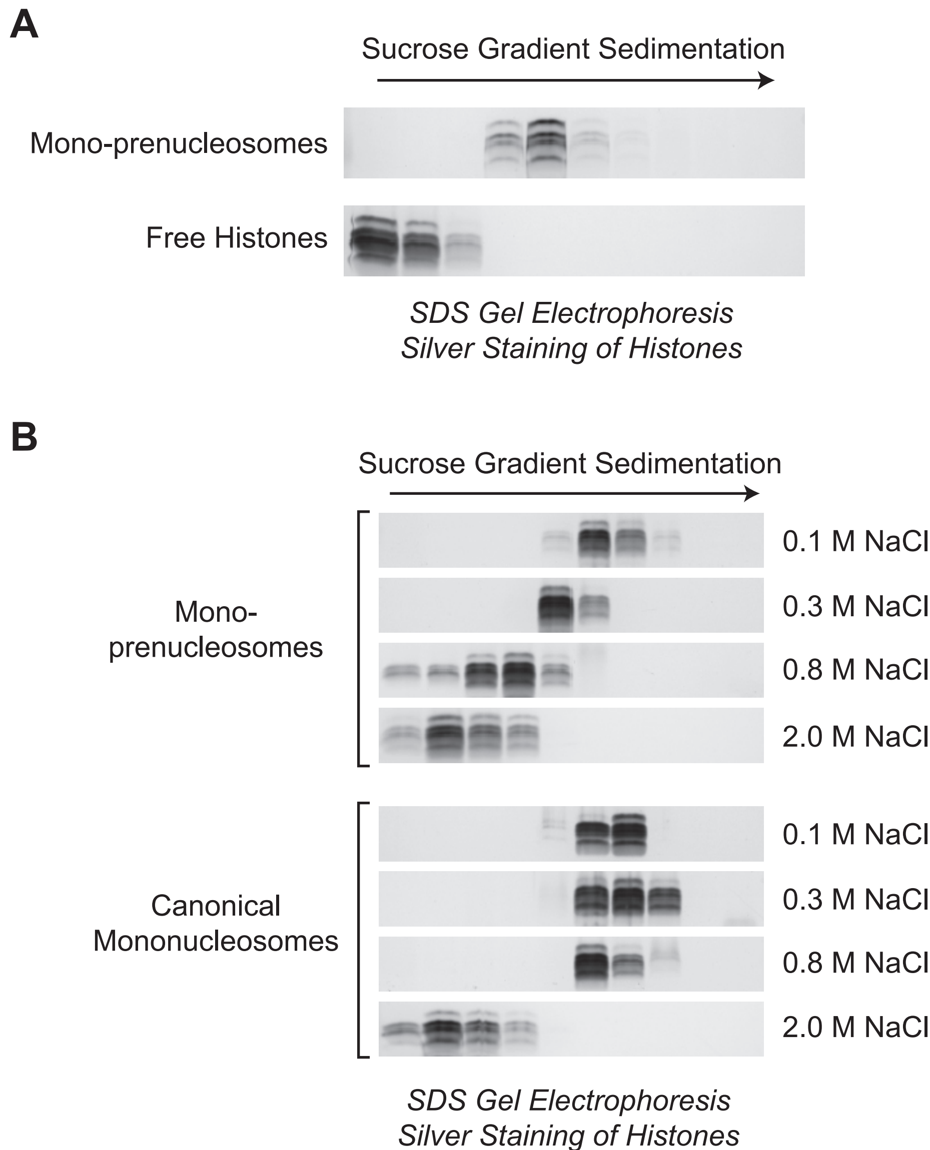
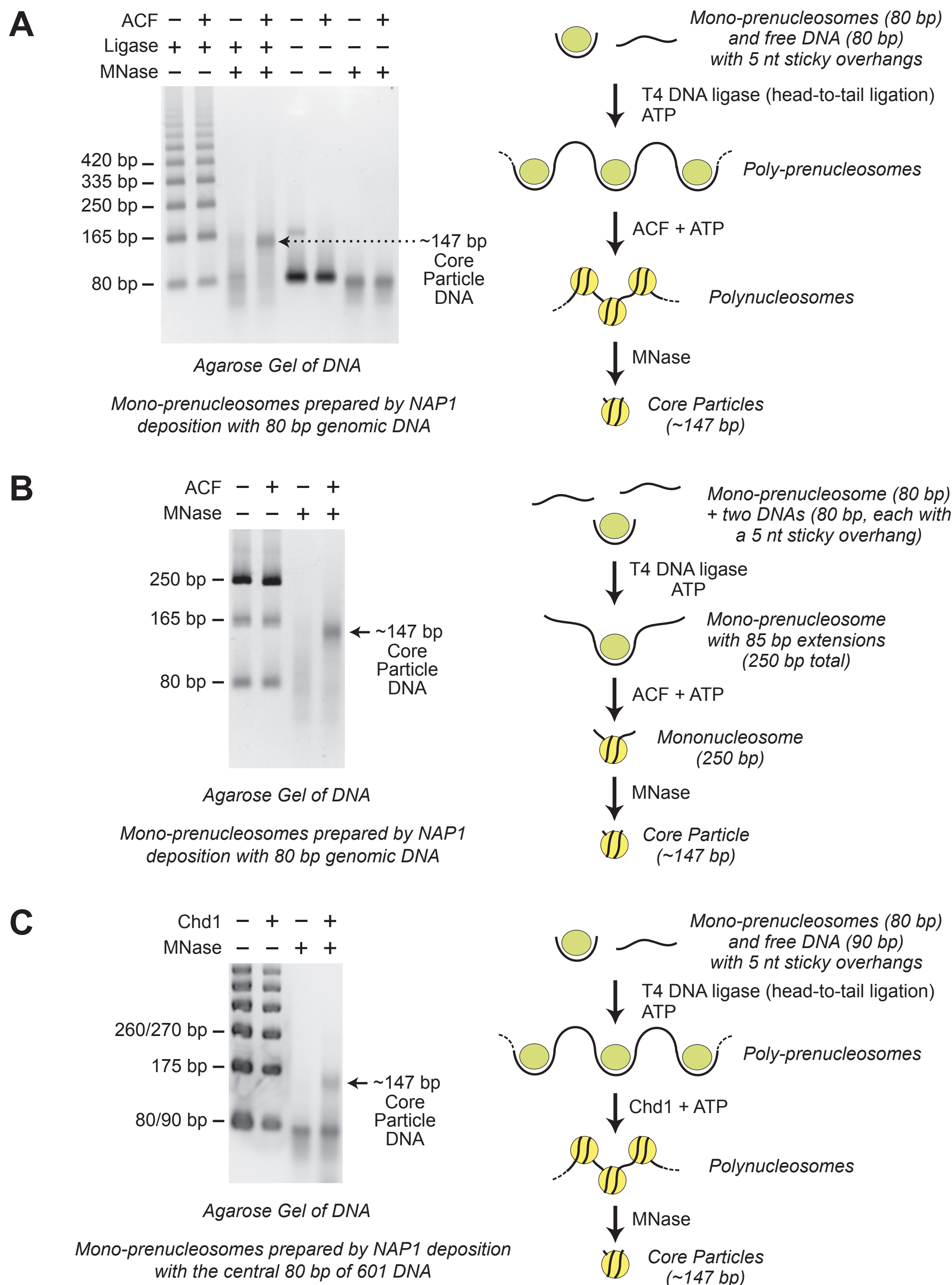


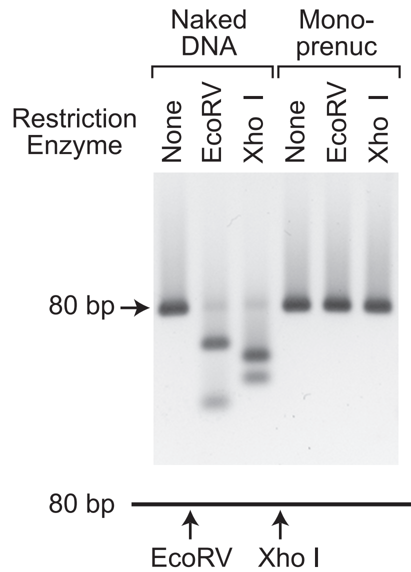
Supplemental Figure S1. The number of psoralen bubbles with prenucleosome templates (−ACF) is approximately the same as the number of bubbles with nucleosome templates (+ACF). The number of psoralen bubbles per plasmid DNA molecule was determined for the prenucleosome and nucleosome samples analyzed in Figure 1 of the main text. These data are based on the analysis of a total of 380 prenucleosome-containing molecules (−ACF) and 376 nucleosome-containing molecules (+ACF) in four independent experiments.



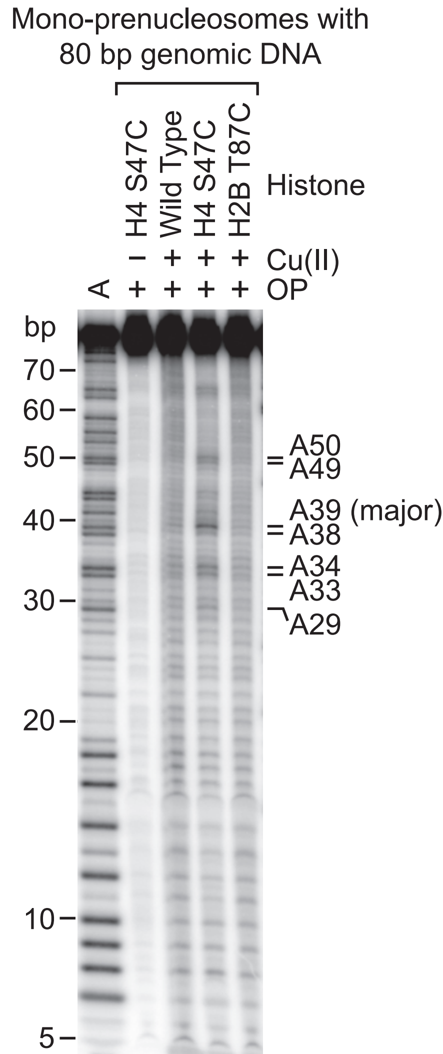
Supplemental Figure S2. Further characterization of mono-prenucleosomes by sucrose gradient sedimentation. (**A**) Mono-prenucleosomes sediment faster than free core histones. Mono-prenucleosomes were reconstituted with NAP1 and the 80 bp genomic DNA. The resulting prenucleosomes were then subjected to 10 to 30% (w/v, left to right) sucrose gradient sedimentation alongside the free core histones in a Beckman SW41 rotor (32,000 rpm, 4˚C, 18 h). The presence of the histones was determined by SDS-polyacrylamide gel electrophoresis and silver staining. (**B**) Mono-prenucleosomes are more salt labile than canonical nucleosomes. Mono-prenucleosomes were reconstituted by salt dialysis to a final concentration of 0.05 M NaCl with the 80 bp genomic DNA. Canonical mononucleosomes were reconstituted by salt dialysis to a final concentration of 0.05 M NaCl with the 146 bp 601 DNA. For each sample shown, the NaCl concentration was adjusted to the indicated amount by the addition of 5 M NaCl. Then, the samples were allowed to incubate at room temperature for 10 min and then subjected to 10 to 30% (w/v, left to right) sucrose gradient sedimentation at the same NaCl concentrations in a Beckman SW55 rotor (32,000 rpm, 4˚C, 17 h). The presence of the histones was determined by SDS-polyacrylamide gel electrophoresis and silver staining. The partial destabilization of the reconstituted canonical nucleosomes at 0.8 M NaCl is similar to that described previously with native nucleosomes (see, for example, Germond et al. 1976). The arrows indicate the direction of sedimentation.



Supplemental Figure S3. Mono-prenucleosomes can be converted into canonical nucleosomes by ACF or Chd1. (**A**) ACF-catalyzed assembly of poly-prenucleosomes to polynucleosomes. Mono-prenucleosomes (prepared by NAP1-mediated deposition with 80 bp genomic DNA) were ligated to free DNA to give poly-prenucleosomes, and then assembled into polynucleosomes with ACF, as in Fig. 4A of the main text. (**B**) ACF-mediated assembly of mono-prenucleosomes to mononucleosomes. Mono-prenucleosomes (prepared by NAP1-mediated deposition with 80 bp genomic DNA) were ligated to free DNA and then assembled into mononucleosomes with ACF, as in Fig. 4B of the main text. (**C**) Chd1-catalyzed assembly of poly-prenucleosomes to polynucleosomes. Mono-prenucleosomes (prepared by NAP1-mediated deposition with the central 80 bp of 601 DNA) were ligated to free DNA to give poly-prenucleosomes, and then assembled into polynucleosomes with Chd1. The formation of canonical nucleosomes was verified by MNase digestion of the polynucleosomes into core particles (~147 bp DNA).



Supplemental Figure S4. Mono-prenucleosomes can block the access of restriction enzymes to DNA. Mono-prenucleosomes were reconstituted by NAP1 deposition with a variant of the 80 bp genomic DNA fragment that contains EcoRV and XhoI restriction sites. The center of the EcoRV restriction site is 25 and 55 bp from the ends of the 80 bp DNA fragment, whereas the center of the XhoI restriction site is 46 and 34 bp from the ends of the DNA. The mono-prenucleosomes were digested with the indicated enzymes along with the corresponding naked DNA fragments. The resulting DNA fragments were analyzed by agarose gel electrophoresis and staining with ethidium bromide.



Supplemental Figure S5. Mapping of the histone-DNA contacts in mono-prenucleosomes assembled with 80 bp of genomic DNA. Core histones containing the wild-type or the indicated mutant histones were modified with N-(1,10-phenanthrolin-5-yl)iodoacetamide (OP), which links an o-phenanthroline moiety onto the histones via alkylation of the thiol group on cysteine residues. The resulting derivatized histones were reconstituted by salt dialysis into mono-prenucleosomes with 80 bp genomic DNA that is ^{32}P-labeled at the 5' end. The hydroxyl radical cleavage reactions were initiated by the addition of Cu(II), hydrogen peroxide, and mercaptopropionic acid. The cleavage products were purified and analyzed by electrophoresis on a 10% polyacrylamide-urea gel. An A-specific chemical sequencing reaction was used to identify the OP cleavage products, which are indicated at the right side of the autoradiogram. H4 S47C is located near the nucleosome dyad.

Supplemental Table S1. Oligonucleotides Used in this Study.

ISWI-80bp (Figs. 2, 3, S2, and S5)	Forward	ATG TCC AAA ACA GAT ACA GCT GCC GTG GAG GCA ACC GAA GAG AAC TCG AAC GAG ACG ACT TCA GAT GCG GCC ACC AGT TC
	Reverse	GAA CTG GTG GCC GCA TCT GAA GTC GTC TCG TTC GAG TTC TCT TCG GTT GCC TCC ACG GCA GCT GTA TCT GTT TTG GAC AT
601-80bp (Figs. 2B and 6)	Forward	TCG TAG ACA GCT CTA GCA CCG CTT AAA CGC ACG TAC GCG CTG TCC CCC GCG TTT TAA CCG CCA AGG GGA TTA CTC CCT AG
	Reverse	CTA GGG AGT AAT CCC CTT GGC GGT TAA AAC GCG GGG GAC AGC GCG TAC GTG CGT TTA AGC GGT GCT AGA GCT GTC TAC GA
ISWI-84bp-RE (Fig. S4)	Forward	CTA GAT TAC ATA TGT CCA AAA CAG AAG ATA TCG CCG TGG AGG CAA CCC TCG AGA ACT CGA ACG AGA CGC CAT GGG ATG CGG CCC
	Reverse	CCG GGG GCC GCA TCC CAT GGC GTC TCG TTC GAG TTC TCG AGG GTT GCC TCC ACG GCG ATA TCT TCT GTT TTG GAC ATA TGT AAT
ISWI-80-AN (Figs. 4A, S3A, and S3B)	Forward	/5Phos/CAT TCA TGT CCA AAA CAG ATA CAG CTG CCG TGG AGG CAA CCG AAG AGA ACT CGA ACG AGA CGA CTT CAG ATG CGG CCA CCA GTT CTT CCG
	Reverse	/5Phos/GAA CTG GTG GCC GCA TCT GAA GTC GTC TCG TTC GAG TTC TCT TCG GTT GCC TCC ACG GCA GCT GTA TCT GTT TTG GAC AT
ISWI-80-AN flank A (Figs. 4B, 5, and S3B)	Forward	/5Phos/ATC CGG TGA AAA GGA GGC TGA GTT CGA CAA CAA AAT CGA GGC TGA TCG CAG TAG GCG CTT TGA TTT CCT GCT AAA GCA GAC ATT C
	Reverse	GAA TGT CTG CTT TAG CAG GAA ATC AAA GCG CCT ACT GCG ATC AGC CTC GAT TTT GTT GTC GAA CTC AGC CTC CTT TTC ACC GGA TCG GAA
ISWI-80-AN flank B (Figs. 4B, 5, and S3B)	Forward	CGG AGA TAT TCA CCC ACT TCA TGA CTA ACA GCG CTA AGA GTC CCA CGA AGC CTA AGG GTA GAC CCA AGA AGA TCA AAG AC
	Reverse	/5Phos/GAA TGG TCT TTG ATC TTC TTG GGT CTA CCC TTA GGC TTC GTG GGA CTC TTA GCG CTG TTA GTC ATG AAG TGG GTG AAT ATC TCC G
ISWI-80bp-AN 90bp linker (Figs. 4A and S3C)	Forward	/5Phos/ATC CGG TGA AAA GGA GGC TGA GTT CGA CAA CAA AAT CGA GGC TGA TCG CAG TAG GCG CTT TGA TTT CCT GCT AAA GCA GAC GGA GAT ATT
	Reverse	/5Phos/GAA TGA ATA TCT CCG TCT GCT TTA GCA GGA AAT CAA AGC GCC TAC TGC GAT CAG CCT CGA TTT TGT TGT CGA ACT CAG CCT CCT TTT CAC CGG ATC GGA A
ISWI-80bp-AN 80bp linker (Fig. S3A)	Forward	/5Phos/ATC CGG TGA AAA GGA GGC TGA GTT CGA CAA CAA AAT CGA GGC TGA TCG CAG TAG GCG CTT TGA TTT CCT GCT AAA GCA GA
	Reverse	/5Phos/GAA TGT CTG CTT TAG CAG GAA ATC AAA GCG CCT ACT GCG ATC AGC CTC GAT TTT GTT GTC GAA CTC AGC CTC CTT TTC ACC GGA TCG GAA

601-80bp-AN (Figs. 4B, 5, and S3C)	Forward	/5Phos/CAT TCT CGT AGA CAG CTC TAG CAC CGC TTA AAC GCA CGT ACG CGC TGT CCC CCG CGT TTT AAC CGC CAA GGG GAT TAC TCC CTA GTT CCG
	Reverse	/5Phos/CTA GGG AGT AAT CCC CTT GGC GGT TAA AAC GCG GGG GAC AGC GCG TAC GTG CGT TTA AGC GGT GCT AGA GCT GTC TAC GA
Primers for Primer extension (Fig. 5)	Watson strand	TCG TAG ACA GCT CTA GCA CCG C
	Crick strand	CTA GGG AGT AAT CCC CTT GGC G

Supplemental Materials and Methods

Analysis of mono-prenucleosome composition by Strep-H2A pulldown

Mono-prenucleosomes were reconstituted with recombinant core histones onto the 80 bp genomic DNA by NAP1-mediated histone deposition. The core histones were either the wild-type histones, as a control, or a mixture of wild-type histones and Strep-H2A at a 3:1 ratio of H2A:Strep-H2A, with equimolar amounts of H2A+Strep-H2A:H2B:H3:H4. Mono-prenucleosomes (with or without Strep-H2A) were incubated for 2 h at 4˚C with Streptavidin-coupled Dynabeads (Life Technologies) in HEG buffer containing 0.1 M KCl. The beads were washed and then eluted with SDS sample buffer. The resulting samples were subjected to SDS-polyacrylamide gel electrophoresis, and the histones were visualized either by silver staining or by western blot analysis with antibodies against H2A.

Mapping of prenucleosomal DNA by primer extension analysis

DNA sequencing ladders were generated by using the Sequenase version 2.0 DNA sequencing kit (USB) with ^{32}P-labeled primers. For DNA sequencing, the fully ligated DNA (250 bp) was PCR amplified, purified, and used as template DNA. For primer extension analyses, the MNase digestion products were used as the template DNA. Primer extension reactions were performed with *E. coli* DNA polymerase I, large (Klenow) fragment (NEB).

Mapping of histone-DNA contacts in the prenucleosome

The labeling of cysteine residues in histones with N-(1,10-phenanthrolin-5-yl)iodoacetamide (OP; Biotium) and the OP-directed cleavage reactions were performed in a manner similar to that described previously (Brogaard et al. 2012; Henikoff et al. 2014). Some specific details are as follows. Wild-type and mutant (H4S47C or H2BT87C) histone preparations (1 mL; 10 µM histone octamers) were dialyzed at 4˚C overnight against TE containing 2 M NaCl, 10 % (v/v) glycerol, and 0.5 mM tris(2-carboxyethyl)phosphine (TCEP). For each set of core histones, the

histones (150 µL; 10 µM histone octamers) and 5 mM TCEP (3 µL) were combined and incubated for 10 min at 22˚C. Next, 4.5 mM OP in DMSO (1 µL) was added to the histone mixture. The reaction was allowed to proceed for 2 h at 22˚C and overnight at 4˚C, and was then terminated by the addition of 2-mercaptoethanol (0.5 µL; 98% purity). The OP-modified histones were dialyzed overnight at 4˚C against TE buffer containing 2 M NaCl, 5 mM 2-mercaptoethanol, and 10% (v/v) glycerol.

The cleavage reactions were performed as follows. Mono-prenucleosomes (reconstituted with OP-labeled histones and 80 bp DNA fragments that were 5'-^{32}P-labeled in the forward strand (Table S1); 1.3 µM mono-prenucleosomes; 40 µL) were combined with Mapping Buffer (40 µL) [0.1 M Tris-HCl (pH 7.5), 5 mM NaCl, and 0.3 mM $CuSO_4$], 60 mM mercaptoproprionic acid (20 µL), 60 mM hydrogen peroxide (20 µL), and Buffer C (80 µL) [50 mM Tris-HCl (pH 7.5), 2.5 mM NaCl] to give a final volume of 200 µL. The reactions were carried out for 20 min at 22˚C, and were terminated by the addition of 25 mM neocuproine (40 µL). The samples were deproteinized, precipitated with ethanol, and analyzed by 10% polyacrylamide-urea gel electrophoresis.

Supplemental References

Brogaard K, Xi L, Wang JP, Widom J. 2012. A map of nucleosome positions in yeast at base-pair resolution. *Nature* **486**: 496-501.

Germond JE, Bellard M, Oudet P, Chambon P. 1976. Stability of nucleosomes in native and reconstituted chromatins. *Nucleic Acids Res* **3**: 3173-3192.

Henikoff S, Ramachandran S, Krassovsky K, Bryson TD, Codomo CA, Brogaard K, Widom J, Wang JP, Henikoff JG. 2014. The budding yeast Centromere DNA Element II wraps a stable Cse4 hemisome in either orientation in vivo. *eLife* **3**: e01861.