SUPPLEMENTAL DATA

A Simple and Versatile System for the ATP-dependent Assembly of Chromatin

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Supplemental Figures S1-S3
Supplemental Table S1

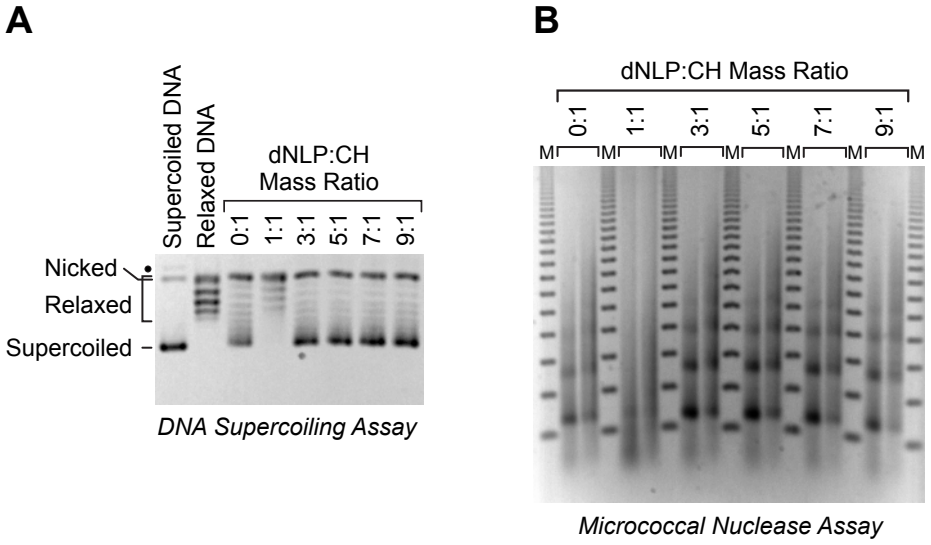

Figure S1. Determination of the optimal dNLP to core histone mass ratio for chromatin assembly. Reactions were performed as in Fig. 1 of the main text with the indicated dNLP to core histone mass ratios. *A*, DNA supercoiling analysis. *B*, Partial MNase digestion analysis. Based on these results, a 5:1 mass ratio of dNLP to core histone octamers (which corresponds to roughly a 6:1 molar ratio of dNLP pentamers to histone octamers) was used throughout this work unless stated otherwise. The low efficiency of chromatin assembly at a 1:1 mass ratio of dNLP to core histones was consistently and reproducibly observed. The basis for this effect is not known.

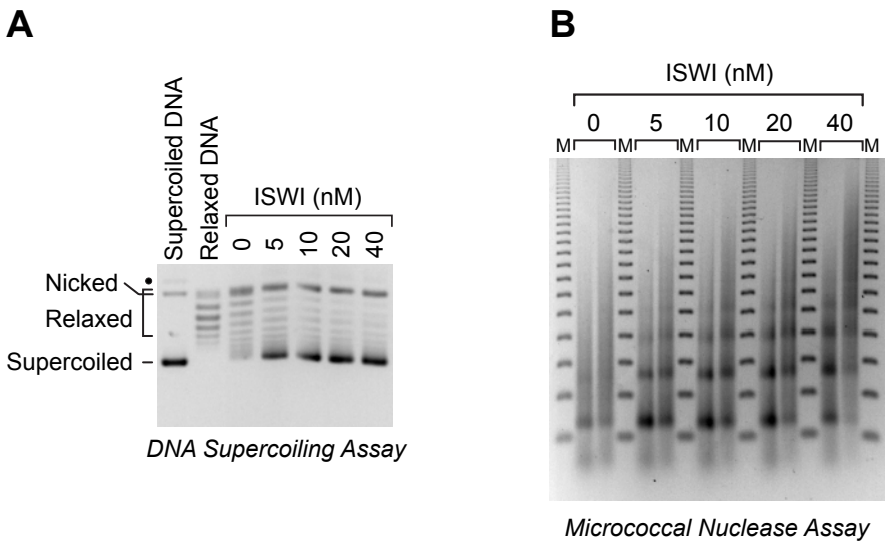

Figure S2. Determination of the optimal ISWI concentration for chromatin assembly. Reactions were performed as in Fig. 1 of the main text with the indicated concentrations of ISWI. *A*, DNA supercoiling analysis. *B*, Partial MNase digestion analysis. Based on these results, a concentration of 20 nM ISWI was used throughout this work unless stated otherwise.

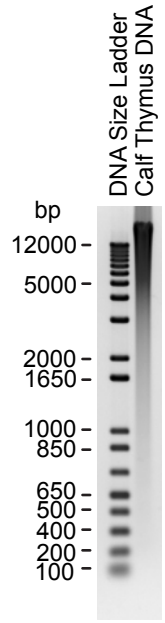

Figure S3. Agarose gel electrophoresis of calf thymus genomic DNA. Purified, unsheared calf thymus DNA (Millipore-Sigma; D4764-1UN) was analyzed by 0.8% agarose gel electrophoresis and staining with ethidium bromide.

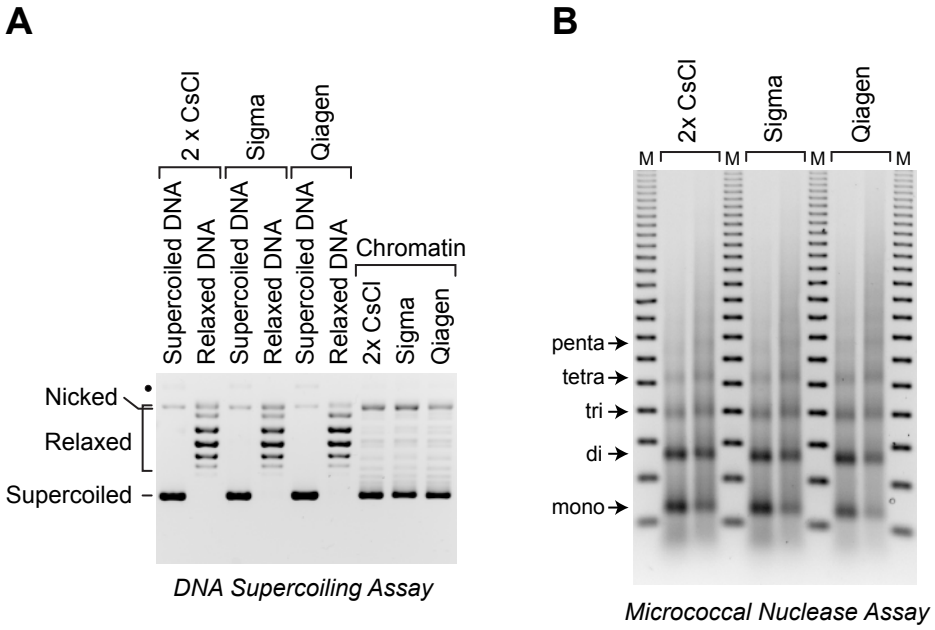

Figure S4. Chromatin can be assembled with DNA prepared by different methods. Chromatin assembly reactions were performed as in Fig. 3 of the main text with plasmid pGIE-0 that was purified by three different methods: (1) two successive CsCl density gradient centrifugation steps; (2) the GenElute HP Plasmid Maxiprep Kit (Millipore Sigma; cat. no. NA0310); and (3) the HiSpeed Plasmid Maxi Kit (Qiagen; cat. no. 12662). *A*, DNA supercoiling analysis. The reaction products were deproteinized, subjected to 0.8% agarose gel electrophoresis, and stained with ethidium bromide. For each method of DNA purification, samples of supercoiled DNA and relaxed DNA were included as references. The positions of nicked DNA, relaxed DNA, and supercoiled DNA are shown. The black dot corresponds to a minor unknown contaminant, which may be supercoiled dimeric plasmid DNA. *B*, Partial MNase digestion analysis. The reaction products were partially digested with two different concentrations of MNase, deproteinized, and subjected to 1.3% agarose gel electrophoresis. The resulting DNA fragments were detected by staining with ethidium bromide. The DNA bands that correspond to mono-, di-, tri-, tetra-, and penta-nucleosomes are shown. The DNA size markers (M) are the 123 bp ladder (Invitrogen).

Table S1. Coding sequences in bacterial expression plasmids

pET21-His6dNLP	ATG**CACCACCACCACCACCAC**CCCATGGCTGAGGAATCATTCTACGGAGTCACTTTGAC CGCCGAGAGCGACAGCGTCACGTGGGATGTAGACGAGGACTACGCACGCGGCCAGAAGC TGGTCATCAAACAGATCCTCTTGGGCGCCGAGGCCAAGGAAAACGAGTTCAACGTGGTC GAGGTGAACACACCCAAGGACTCCGTGCAAATTCCCATCGCCGTATTGAAGGCCGGAGA GACCCGCGCCGTCAATCCCGACGTGGAGTTCTACGAGTCGAAGGTGACGTTCAAGCTGA TCAAGGGCAGCGGACCCGTCTACATCCACGGGCACAACATCAAGGACGATGTGGAGGTG GTCGACATGGAGGAGGATGACGAGGAGGACGATGTGGCCGAGGACGAGGAGGACGAGCA CCCAAAGAAGCGCGCCAAGATCGAGAACGCCGCCGATGGTAAAAATGCCAAGAACAACA AGAAGAAG**TAATGA**CTCGAG **Histidine tag** *Drosophila* dNLP coding region **Stop codons** Restriction sites
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Drosophila ISWI coding region
Combined Sce VMA intein and chitin binding domain (iCBD) coding region
Stop codon
Restriction sites

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