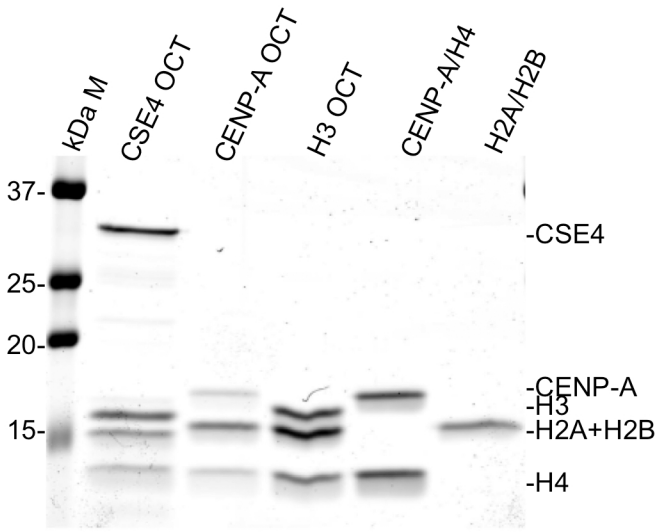
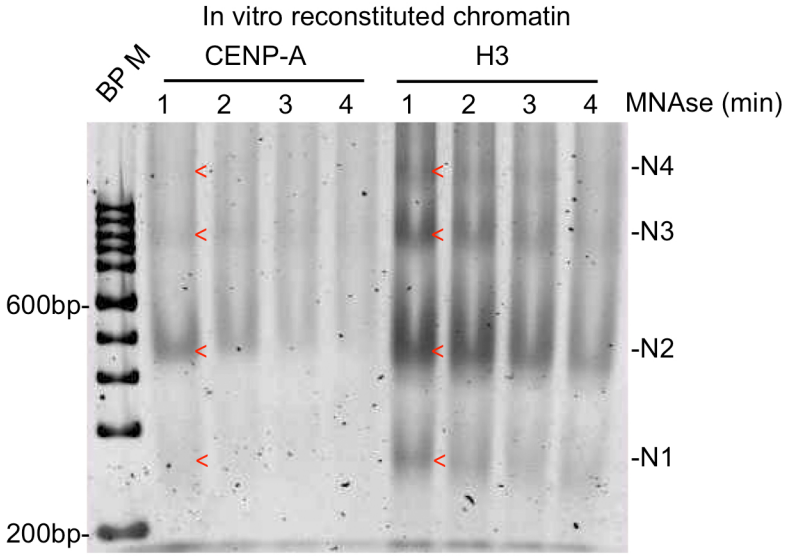


Supplementary Figure 1 Denaturing SDS-PAGE analysis demonstrates equimolar amounts of core histones in each reconstitution.
18% SDS-PAGE protein gel stained with Coomassie Brilliant Blue demonstrates equi-molar amounts of all 4 histones in each in vitro reconstitution (CSE4 OCT, CENP-A OCT, H3 OCT); CENP-A/H4 and H2A/H2B dimers are shown as a reference point.H2A/H2B co-migrate on 18% SDS PAGE gels, and usually separate as a double intensity band distinct from H3, CENP-A and H4.



Supplementary Figure 2. Octameric CENP-A and H3 nucleosomes migrate equivalently on native PAGE gels. 4.5% Native PAGE gel stained with GelStar demonstrates that in vitro reconstitutedCENP-A mono- (N1), di- (N2), tri- (N3), and tetra- (N4) nucleosomes migrate equivalently to their counterpart H3 species on the same gel. Nucleosomal species are marked with a red side arrow for easy reference. 100bp ladder is provided for reference. Mononucleosomes typically migrate between 250bp and 300bp on 4.5% PAGE in 0.5X TBE at 4mAmps.

Supplementary Note

All methods are published in previous work. Salt dialysis follows Noll and colleagues[1], and Dalal and Stein[2]. Briefly, lyophilized recombinant histones (a gift from Jennifer Ottesen) were unfolded in 7M guanidinium HCl, mixed in equimolar amounts (Either H3 or CENP-A and one each H2A, H2B, H4), and refolded into 2M NaCl according to the protocol by Luger et al.[3], or purified histone octamers (a gift from Aaron Straight, Takehito Furuyama, and Jennifer Gerton) were reconstituted onto a plasmid containing "Widom 601" positioning sequence (a gift from Carl Wu) or centromeric alpha-satellite repeat containing plasmid (a gift from Delphine Quenet) using sequential salt dialysis adapted for low volumes[11]. Histone octamers were mixed with plasmid DNA at 0.9:1 ratio in 2M NaCl, 10mM Tris-Cl pH=8.0, 1mM EDTA (0.18 mg/ml histones; 0.2 mg/ml DNA) and incubated on ice for 30 min. 40 ul of histone/DNA mix was layered onto a dialysis disc (Millipore, 0.025um) covered with a dialysis membrane (Thermo Scientific, 7000 MWCO) and floated on the surface of 50 ml pre-chilled 1M NaCl, 10mM Tris-Cl pH=8.0, 1mM EDTA buffer. Sequential dialysis steps against 1M, 0.8M, 0.6M, and 0.15M NaCl (each with 10mM Tris-Cl pH=8.0, 1mM EDTA) were carried out for 2 hours at 4 deg (0.6M dialysis was done overnight). Light MNase digestion of a fraction of the reconstituted chromatinized plasmids was performed (1unit Sigma MNase/ug of chromatin for 1, 2 or 4 minutes at 37C) to release CENP-A- and H3- mono, di, and trinucleosomes, which were promptly analyzed by SDS-PAGE and native PAGE[4]. Native PAGE: briefly 4.5% PAGE gels were electrophoresed at 4mAmps for 3hrs at 4C in 0.25-0.5X TBE running buffer- mononucleosomes generally migrate with the Xylene Cyanol band. The remaining fraction of reconstituted chromatin samples were diluted 100-fold in 1X PBS, 2mM $MgCl_2$ buffer and imaged by AFM on freshly prepared APS- or APTES-mica following standardized protocols routinely used in our laboratory[4]. H3- and CENP-A samples were always measured on the same day, using the same cantilever, to account for variations in humidity and other environmental conditions in parallel. AFM was performed in tapping mode on the Agilent 5500 AFM.

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10.1093/nar/gki224 (2005).

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