

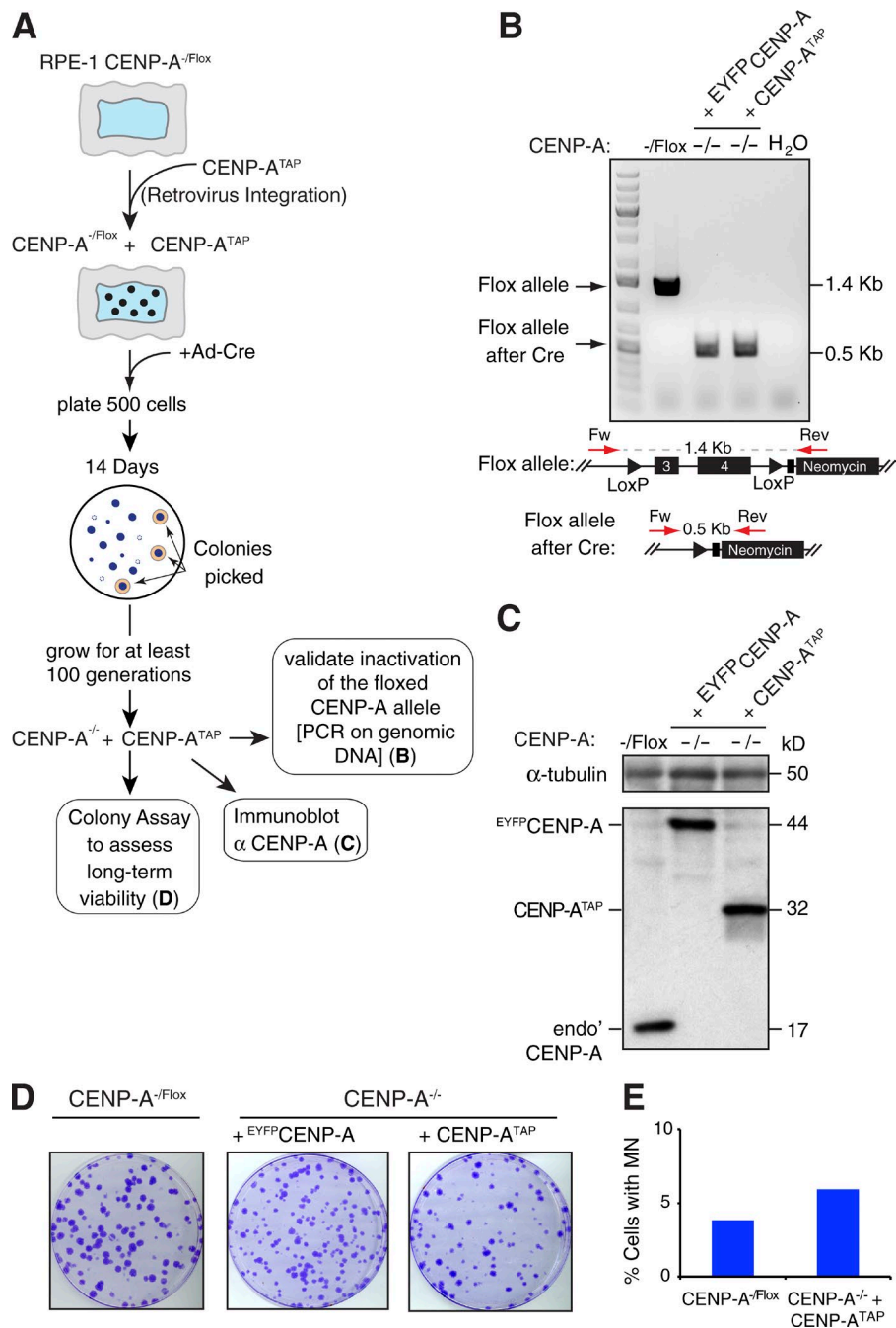
Nechemia-Arbely et al., <https://doi.org/10.1083/jcb.201608083>

Figure S1. **CENP-A^{TAP} maintains long-term centromere function and viability in the absence of endogenous CENP-A.** (A) Schematic of cell line construction and of the experiments in B–D. (B) PCR analysis on the DNA extracted from surviving colonies after >100 generations of growth after adeno-Cre treatment to inactivate the remaining CENP-A allele in CENP-A^{-/-} RPE-1 cells expressing EYFP-CENP-A or CENP-A^{TAP}. (C) Immunoblot to visualize levels of each CENP-A-tagged rescue construct or endogenous (endo') CENP-A. α-Tubulin was used as a loading control. (D) Images of representative crystal violet-stained colonies from a colony formation assay for measuring long-term viability. (E) Micronuclei (MN) quantification in CENP-A^{-/-} RPE-1 and after adeno-Cre treatment to inactivate the remaining CENP-A allele in CENP-A^{-/-} RPE-1 cells expressing CENP-A^{TAP}.

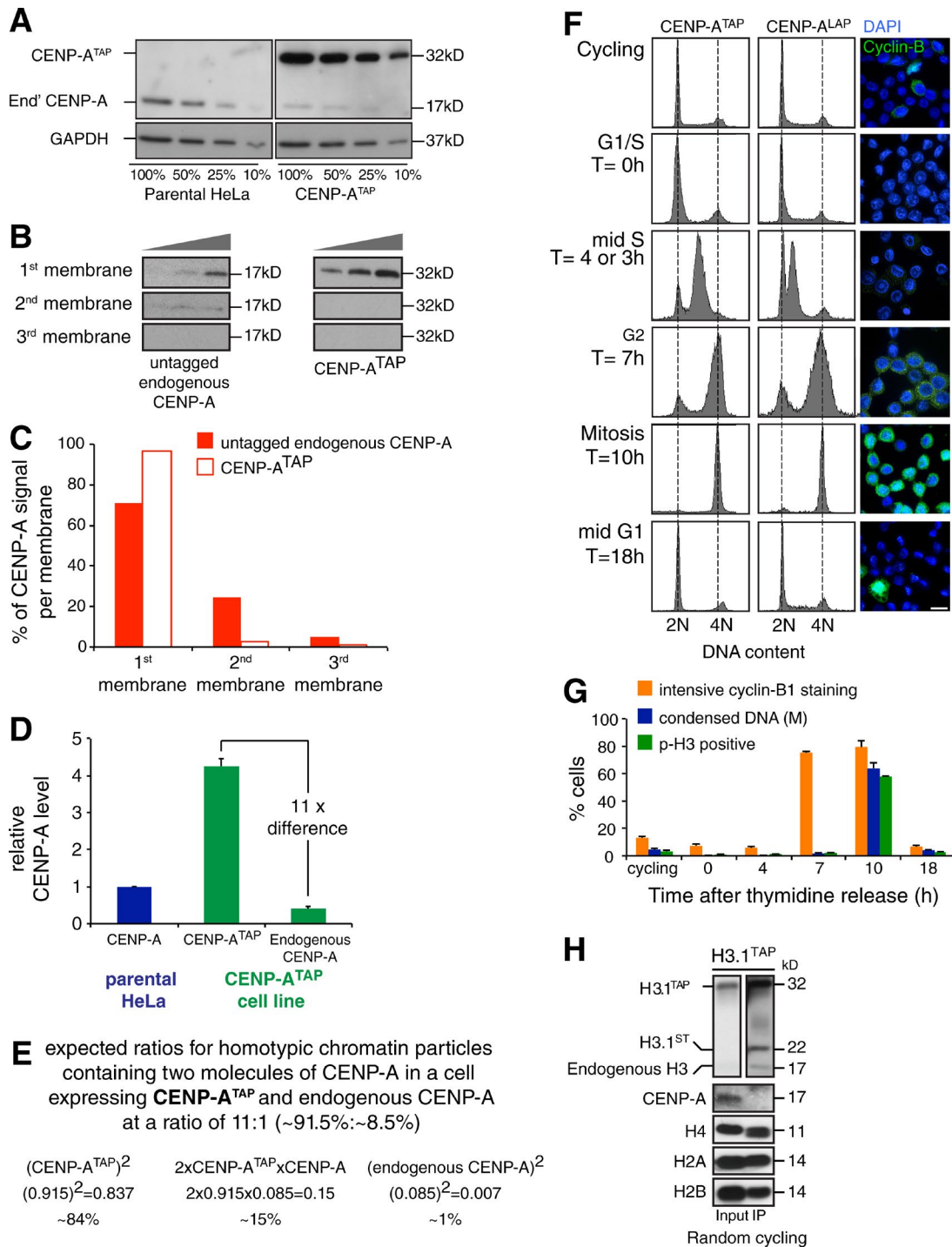
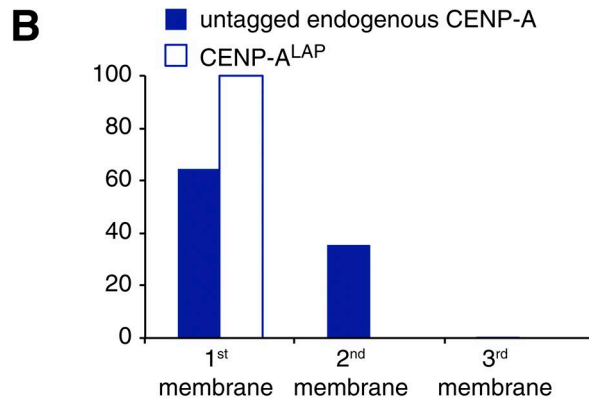
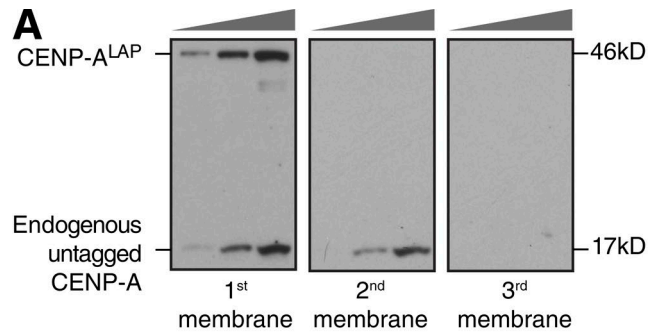


Figure S2. **Characterization of CENP-A^{TAP} cells and synchronization efficiency for producing CENP-A chromatin at multiple cell cycle points.** (A) Immunoblot for CENP-A in whole-cell extracts of a parental HeLa cell line and a CENP-A^{TAP}-expressing cell line. (B) Serial 1:2 dilutions of whole-cell extracts of CENP-A^{TAP}-expressing cells, immunoblotted for CENP-A, after protein transfer onto a stack of three membranes. (C) Quantification of the fraction of CENP-A retained on the first membrane (compared with the total signal from all three membranes) shown in B. (D) Quantification of CENP-A expression levels (tagged and endogenous) shown in A after correcting for the fraction of CENP-A that passes through the immunoblot membrane (as shown in B and C). $n = 4$ from four independent loadings in the gel shown in A. Error bars represent SEM. (E) Calculation of the expected ratios of homotypic chromatin particles containing two molecules of CENP-A in a cell expressing CENP-A^{TAP} and endogenous CENP-A at a ratio of 11:1, respectively. (F) Synchronization efficiency of CENP-A^{TAP} and CENP-A^{ALAP} cells monitored by FACS analysis of DNA content and cyclin B immunofluorescence. Bar, 20 μm . (G) Quantification of cyclin B immunofluorescence, p-H3-positive cells, and cells with condensed DNA at different time points after the release of CENP-A^{TAP} cells from a second thymidine block (G1/S point). $n = 6$ from six independent experiments. Error bars represent SEM. (H) Affinity purification of H3.1^{TAP} results in coimmunoprecipitation of endogenous H3, H4, H2A, and H2B. CENP-A is not present within the H3.1^{TAP} mononucleosomes.



C expected ratios for homotypic chromatin particles containing two molecules of CENP-A in a cell expressing endogenously tagged **CENP-A^{LAP}** and non-tagged CENP-A at a ratio of 1:1

(CENP-A ^{LAP}) ²	2xCENP-A ^{LAP} xCENP-A	(Non tagged CENP-A) ²
(0.5) ² =0.25	2x0.5x0.5=0.5	(0.5) ² =0.25
25%	50%	25%

Figure S3. **Transfer efficiency of untagged endogenous CENP-A and endogenously tagged CENP-A^{LAP}.** (A) Serial 1:2 dilutions of whole-cell extracts of CENP-A^{LAP}-expressing cells, immunoblotted for CENP-A, after protein transfer onto a stack of three membranes. (B) Quantification of the fraction of CENP-A retained on the first membrane (compared with the total signal from all three membranes) shown in A. (C) Calculation of the expected ratios of homotypic chromatin particles containing two molecules of CENP-A in the cell line CENP-A^{+/LAP}, expressing CENP-A^{LAP} and nontagged CENP-A at a ratio of 1:1 as shown in Fig. 4 (D and E).

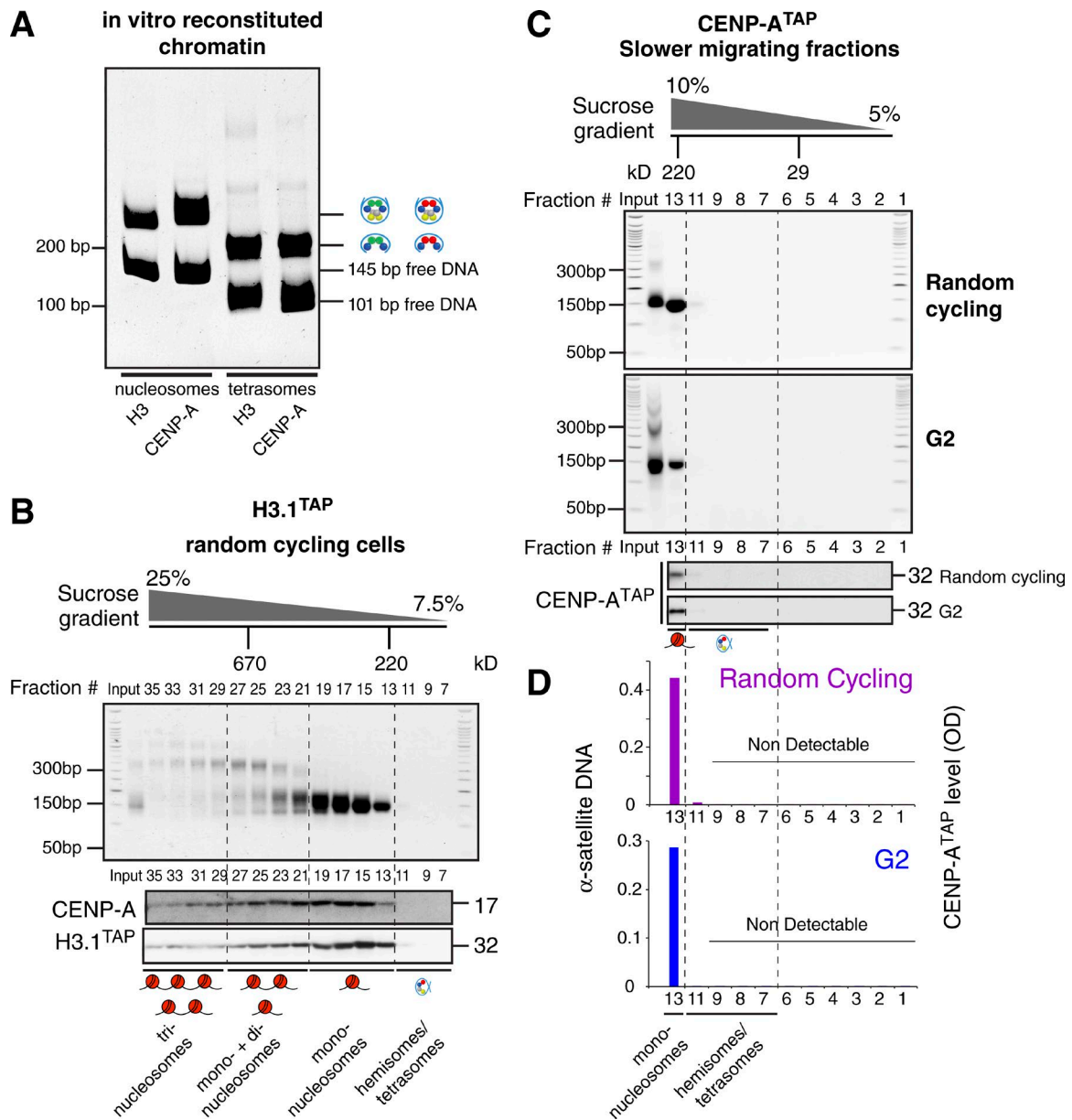


Figure S4. **Sedimentation of CENP-A is inconsistent with a hemisome/tetrasome structure as the major form of CENP-A chromatin.** (A) Ethidium bromide-stained 5% native PAGE of in vitro-reconstituted octameric H3 and CENP-A nucleosome wrapped with 145 bp of template DNA or (CENP-A/H4)₂ or (H3/H4)₂ tetrasomes wrapped with 101 bp of template DNA. (B) Sedimentation velocity of chromatin in random cycling H3.1^{TAP} cells. (top) DNA length in the different fractions. (bottom) Immunoblotting for CENP-A and H3. (C, top) Ethidium bromide-stained agarose gel of DNA extracted from the slower-migrating chromatin fractions from randomly cycling or G2 CENP-A^{TAP} cells. (bottom) Immunoblot for CENP-A. (D) Real-time quantitative PCR with primers for α-satellite DNA.

Table S1. Read statistics for ChIP-sequencing experiments

Experiment	Replicate number	Total number of merged paired-end reads (100 bp × 2)	Total number of merged reads ≥100 bp	Number of merged reads mapping to α-satellites
			%	%
CENP-A ^{TAP} G1	1	30,632,549	29,112,562 (95%)	12,716,461 (43.6%)
	2	25,982,533	22,232,953 (85.5%)	9,836,077 (44.2%)
CENP-A ^{TAP} G2	1	24,210,941	21,834,136 (90.2%)	11,929,448 (54.6%)
	2	29,836,502	26,273,103 (88.1%)	15,450,060 (58.9%)
H3.1 ^{TAP} RC	1	31,819,170	30,928,233 (97.2%)	668,075 (2.1%)
	2	42,069,089	40,218,049 (95.6%)	911,027 (2.2%)
CENP-A ^{TAP} RC input	1	61,557,119	61,434,004 (99.8%)	1,374,644 (2.2%)

Total number of merged paired-end reads (one read per merged two paired ends) generated for each sample, the number (and percentage) of those that were ≥100 bp in length, and the number (and percentage) of reads mapping to α-satellites. RC, random cycling.

Table S2. ChIP sequencing combined replicate statistics

Experiment	Total number of merged paired-end reads (100 bp × 2)	Total number of merged reads ≥100 bp	Number of merged reads mapping to α-satellites
		%	%
CENP-A ^{TAP} G1	56,615,082	51,345,515 (90.7%)	22,552,538 (43.9%)
CENP-A ^{TAP} G2	54,047,443	48,107,239 (89%)	27,379,508 (56.8%)
H3.1 ^{TAP} RC	73,888,259	71,146,282 (96.3%)	1,579,102 (2.15%)

Read statistics is given for the combined replicates in each condition. This table was used in Fig. 2 D.

Table S3. Human α -satellite arrays in centromeric reference models

hg38 chromosome number	Chromosome coordinates		GenBank accession number	HuRef reference model length	Total array length	Total array length
	Start	End				
chr1	122,026,459	122,224,535	GJ211836.1	198,076		
chr1	122,224,635	122,503,147	GJ211837.1	278,512		
chr1 ^a	122,503,247	124,785,432	GJ212202.1	2,282,185		
chr1	124,785,532	124,849,129	GJ211855.1	63,597		
chr1	124,849,229	124,932,724	GJ211857.1	83,495		
chr1					2,905,865	2.9
chr2	92,188,145	94,090,557	GJ211860.1	1,902,412	1,902,412	1.9
chr3	90,772,458	91,233,586	GJ211866.1	461,128		
chr3	91,233,686	91,247,622	GJ211867.1	13,936		
chr3	91,553,419	93,655,574	GJ211871.1	2,102,155		
chr3					2,577,219	2.6
chr4	49,712,061	51,743,951	GJ211881.1	2,031,890	2,031,890	2.0
chr5	46,485,900	46,569,062	GJ211882.1	83,162		
chr5	46,569,162	46,796,725	GJ211883.1	227,563		
chr5	46,796,825	47,061,288	GJ211884.1	264,463		
chr5	47,106,994	47,153,339	GJ211886.1	46,345		
chr5	47,153,439	47,296,069	GJ211887.1	142,630		
chr5 ^a	47,309,184	49,591,369	GJ212203.1	2,282,185		
chr5 ^b	49,667,531	49,721,203	GJ211904.2	53,672		
chr5	49,721,303	50,059,807	GJ211906.2	338,504		
chr5					3,438,524	3.4
chr6	58,553,888	59,829,934	GJ211907.1	1,276,046	1,276,046	1.3
chr7	58,169,653	60,828,234	GJ211908.1	2,658,581		
chr7	61,377,788	61,528,020	GJ212194.1	150,232		
chr7					2,808,813	2.8
chr8	44,033,744	45,877,265	GJ211909.1	1,843,521	1,843,521	1.8
chr9	43,389,635	45,518,558	GJ211929.1	2,128,923	2,128,923	2.1
chr10	39,686,682	39,935,900	GJ211930.1	249,218		
chr10	39,936,000	41,497,440	GJ211932.1	1,561,440		
chr10	41,497,540	41,545,720	GJ211933.1	48,180		
chr10	41,545,820	41,593,521	GJ211936.1	47,701		
chr10					1,906,539	1.9
chr11	51,078,348	51,090,317	GJ211938.1	11,969		
chr11	51,090,417	54,342,399	GJ211943.1	3,251,982		
chr11	54,342,499	54,425,074	GJ211948.1	82,575	3,346,526	3.3
chr11					3,346,526	3.3
chr12	34,769,407	34,816,611	GJ211949.1	47,204		
chr12	34,835,295	37,185,252	GJ211954.1	2,349,957		
chr12					2,397,161	2.4
chr13 ^c	16,000,000	16,022,537	GJ211955.2	22,537		
chr13 ^c	16,022,637	16,110,659	GJ211961.2	88,022		
chr13 ^c	16,110,759	16,164,892	GJ211962.2	54,133		
chr13 ^c	16,164,992	16,228,527	GJ211963.2	63,535		
chr13 ^c	16,228,627	16,249,297	GJ211965.2	20,670		
chr13 ^c	16,249,397	16,256,067	GJ211967.2	6,670		
chr13 ^c	16,256,167	16,259,412	GJ211968.2	3,245		
chr13 ^c	16,259,512	16,282,073	GJ211969.2	22,561		
chr13 ^c	16,282,173	17,416,384	GJ211972.2	1,134,211		
chr13 ^c	17,416,484	17,416,824	GJ212205.1	340		
chr13 ^c	17,416,924	17,417,264	GJ212206.1	340		
chr13 ^c	17,417,364	17,418,562	GJ211986.2	1,198		
chr13 ^c	17,418,662	18,051,248	GJ211991.2	632,586		
chr13					2,050,048	2.1
chr14 ^c	16,000,000	16,022,537	GJ211992.2	22,537		
chr14 ^c	16,140,627	16,228,649	GJ211998.2	88,022		
chr14 ^c	16,228,749	16,282,882	GJ211999.2	54,133		
chr14 ^c	16,282,982	16,346,517	GJ212000.2	63,535		
chr14 ^c	16,346,617	16,367,287	GJ212002.2	20,670		

Table S3. Human α -satellite arrays in centromeric reference models (Continued)

hg38 chromosome number	Chromosome coordinates		GenBank accession number	HuRef reference model length	Total array length	Total array length
	Start	End				
chr14 ^c	16,367,387	16,374,057	GJ212004.2	6,670		
chr14 ^c	16,374,157	16,377,402	GJ212005.2	3,245		
chr14 ^c	16,377,502	16,400,063	GJ212006.2	22,561		
chr14 ^c	16,404,448	17,538,659	GJ212009.2	1,134,211		
chr14 ^c	17,538,759	17,539,099	GJ212210.1	340		
chr14 ^c	17,539,199	17,539,539	GJ212211.1	340		
chr14 ^c	17,539,639	17,540,837	GJ212023.2	1,198		
chr14 ^c	17,540,937	18,173,523	GJ212028.2	632,586		
chr14					2,050,048	2.1
chr15	17,083,673	17,498,951	GJ212036.1	415,278		
chr15	17,499,051	18,355,008	GJ212042.1	855,957		
chr15	18,355,108	19,725,254	GJ212045.1	1,370,146		
chr15					2,641,381	2.6
chr16	36,311,158	36,334,460	GJ212046.1	23,302		
chr16	36,337,666	38,265,669	GJ212051.1	1,928,003		
chr16					1,951,305	2.0
chr17	22,813,679	23,194,918	GJ212053.1	381,239		
chr17	23,195,018	26,566,633	GJ212054.1	3,371,615		
chr17	26,566,733	26,616,164	GJ212055.1	49,431		
chr17					3,802,285	3.8
chr18	15,460,899	15,780,377	GJ212060.1	319,478		
chr18	15,797,855	20,561,439	GJ212062.1	4,763,584		
chr18	20,603,247	20,696,289	GJ212066.1	93,042		
chr18	20,696,389	20,736,025	GJ212067.1	39,636		
chr18	20,736,125	20,813,083	GJ212069.1	76,958		
chr18	20,839,797	20,861,206	GJ212071.1	21,409		
chr18					5,314,107	5.3
chr19 ^b	24,498,980	24,552,652	GJ212072.2	53,672		
chr19	24,552,752	24,891,256	GJ212077.2	338,504		
chr19 ^a	24,908,689	27,190,874	GJ212201.1	2,282,185		
chr19					2,674,361	2.7
chr20	26,436,232	26,586,955	GJ212091.1	150,723		
chr20	26,608,145	28,494,539	GJ212093.1	1,886,394		
chr20	28,508,997	28,556,953	GJ212095.1	47,956		
chr20	28,648,108	28,728,874	GJ212105.1	80,766		
chr20	29,125,793	29,204,668	GJ212107.1	78,875		
chr20	29,917,404	30,038,348	GJ212117.1	120,944		
chr20					2,365,658	2.4
chr21 ^c	10,864,560	10,887,097	GJ212118.2	22,537		
chr21 ^c	10,887,197	10,975,219	GJ212124.2	88,022		
chr21 ^c	10,975,319	11,029,452	GJ212125.2	54,133		
chr21 ^c	11,029,552	11,093,087	GJ212126.2	63,535		
chr21 ^c	11,093,187	11,113,857	GJ212128.2	20,670		
chr21 ^c	11,113,957	11,120,627	GJ212130.2	6,670		
chr21 ^c	11,120,727	11,123,972	GJ212131.2	3,245		
chr21 ^c	11,124,072	11,146,633	GJ212132.2	22,561		
chr21 ^c	11,146,733	12,280,944	GJ212135.2	1,134,211		
chr21 ^c	12,281,044	12,281,384	GJ212204.1	340		
chr21 ^c	12,281,484	12,281,824	GJ212207.1	340		
chr21 ^c	12,281,924	12,283,122	GJ212149.2	1,198		
chr21 ^c	12,283,222	12,915,808	GJ212154.2	632,586		
chr21					2,050,048	2.1
chr22 ^c	12,954,788	12,977,325	GJ212155.2	22,537		
chr22 ^c	13,021,422	13,109,444	GJ212161.2	88,022		
chr22 ^c	13,109,544	13,163,677	GJ212162.2	54,133		
chr22 ^c	13,163,777	13,227,312	GJ212163.2	63,535		
chr22 ^c	13,227,412	13,248,082	GJ212165.2	20,670		
chr22 ^c	13,248,182	13,254,852	GJ212167.2	6,670		
chr22 ^c	13,254,952	13,258,197	GJ212168.2	3,245		

Table S3. Human α -satellite arrays in centromeric reference models (Continued)

hg38 chromosome number	Chromosome coordinates		GenBank accession number	HuRef reference model length	Total array length	Total array length
	Start	End				
chr22 ^c	13,258,297	13,280,858	GJ212169.2	22,561		
chr22 ^c	13,285,243	14,419,454	GJ212172.2	1,134,211		
chr22 ^c	14,419,554	14,419,894	GJ212208.1	340		
chr22 ^c	14,419,994	14,420,334	GJ212209.1	340		
chr22 ^c	14,420,434	14,421,632	GJ212186.2	1,198		
chr22 ^c	14,421,732	15,054,318	GJ212191.2	632,586		
chr22					2,050,048	2.1
chrX	58,605,579	62,412,542	GJ212192.1	3,806,963	3,806,963	3.8

Centromere reference models (unpublished data) were generated with methods as previously described (Miga et al., 2014). Length estimates are expected to be averaged across arrays from homologous chromosomes. Column 1, chromosome information; column 2, chromosome start position; column 3, chromosome end position; column 4, GenBank accession number; column 5, length of each reference model as represented in the human assembly; column 6, total array length (in base pairs); column 7, total array length (in megabases). Sequence coordinates refer to the human Genome Reference Consortium human genome (build 38) assembly.

^aThree near-identical arrays on chromosomes 1, 5, and 19.

^bTwo near-identical arrays on chromosomes 5 and 19.

^cAcrocentric near-identical arrays on chromosomes 13, 14, 21, and 22.

Provided online is Table S4 as a PDF. It shows CENP-A^{TAP} and H3.1^{TAP} sequence mapping onto α -satellite DNA in human centromere reference models for each autosome and the X chromosome.

Reference

Miga, K.H., Y. Newton, M. Jain, N. Altemose, H.F. Willard, and W.J. Kent. 2014. Centromere reference models for human chromosomes X and Y satellite arrays. *Genome Res.* 24:697–707. <http://dx.doi.org/10.1101/gr.159624.113>