CENP-B box, a nucleotide motif involved in centromere formation, occurs in a New World monkey

Electronic supplementary material

Supplementary methods

(Detailed description of the methods used in this study)

Immunofluorescent cell staining: Marmoset epithelial cells and human HeLa cells were cultured in AminoMAX-II Complete Medium (Life technologies) and D-MEM High Glucose (Wako) supplemented with 10% FBS and penicillin-streptomycin, respectively, in a humidified incubator set at 37 °C and 5% CO_2. Immunofluorescence staining was performed as described previously [14] with a few modifications. Cells grown in glass-bottom plates were fixed in 2.6% formaldehyde in PBS for 10 min at room temperature, and quenched with 0.4% Triton X-100 and 100 mM glycine in PBS for 7 min at room temperature. The fixed cells were incubated in blocking solution, 1% BSA in PBS-T (PBS containing 0.1% Triton X-100), for 30 min at room temperature. Primary antibody incubation was performed in 0.1% BSA in PBS-T for 1 h at room temperature and the cells were washed with PBS-T four times. Incubation with fluorescently labeled secondary antibody was performed in 0.1% BSA in PBS-T for 1 hour at room temperature and the cells were twice washed with PBS-T. The cells were then incubated in PBS-T containing DAPI for 5 min at room temperature. After washing with PBS-T and PBS, images were acquired using an LSM700 microscope (Zeiss) equipped with Alpha Plan-Apochromat 63×/1.46 Oil M27 lens (Zeiss). Three slices of Z-stacks with a spacing of 0.34 μm were displayed as maximum intensity projections using Zen 2009 (Zeiss). Signals of small sizes compared to the survey spacing might escape fluorescence detection, possibly resulting in an underestimation of the signal number. The following antibodies were used. For CENP-A staining, rat anti-human CENP-A antibody (6F2 [14]) and goat anti-rat IgG (H+L) secondary antibody, Alexa Fluor 694 conjugate (Life technologies). For CENP-B staining, mouse monoclonal anti-human CENP-B antibody (5E6C1) and goat anti-mouse IgG (H+L)

secondary antibody, Alexa Fluor 488 conjugate (Life technologies). Our anti-human CENP-A antibody and anti-human CENP-B antibody can be thought to recognize marmoset CENP-A and CENP-B, respectively, because the amino acid sequences of marmoset CENP-A and CENP-B exhibit high similarities to their human equivalents: 91.4% and 96.6% identity, respectively [2].

Sequencing of fosmid clones for long contig seqences: The strategy of this method was fully described in our previous reports [13, 18], and its essence is described here. The fosmid clone to be sequenced (FosMar08) contained a 40-kb AS fragment. At the boundary between the vector portion and the insert fragment, there was a recognition site for the restriction endonuclease *Bam*HI. The insert fragment did not contain a *Bam*HI site. We delivered a Tn5 transposon into this fosmid clone by inducing its transposition, and collected several secondary clones. The Tn5 transposon had been modified in advance to carry a *Bam*HI site. This modification enabled us to determine the position of the Tn5 insertion points by digesting the secondary clones with *Bam*HI and measuring the fragment size by gel electrophoresis. From the collection of the secondary clones for which the insertion points were determined, we selected a total of 24 clones so that neighboring clones would be located within 1.0 kb. We then sequenced the selected secondary clones by using a pair of primers that corresponded to the internal region of the Tn5 transposon and were oriented outwards, which resulted in sequence reads of more than 1.5 kb in length. By assembling these sequence reads in the order of their positions, we obtained a contig sequence of 13.1 kb in length.

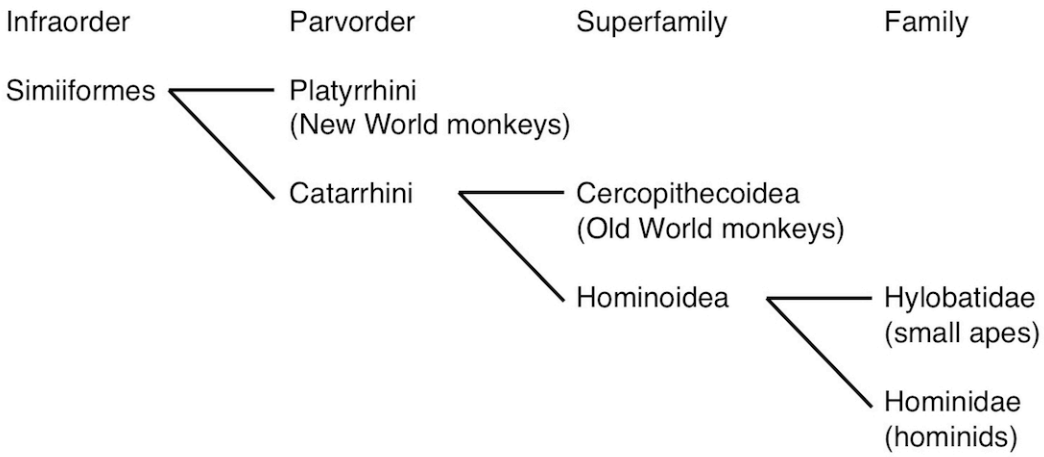

Figure S1. Phylogenetic structure of simian primates. The upper branch of each taxonomic level is further divided into lower-level taxa (not shown).

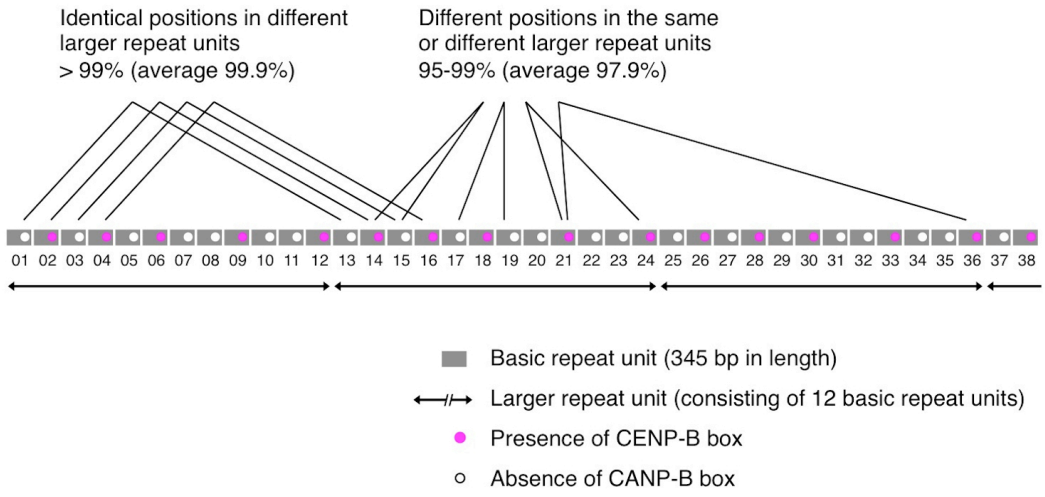

Figure S2. Structure of AS repeat units contained in the FosMar08 clone. The higher-order structure was found in our previous study [12], and sequence identities observed in this clone are shown. It is clear that repetition of a specific distribution pattern of CENP-B boxes coincides with the higher-order repeat structure of AS.

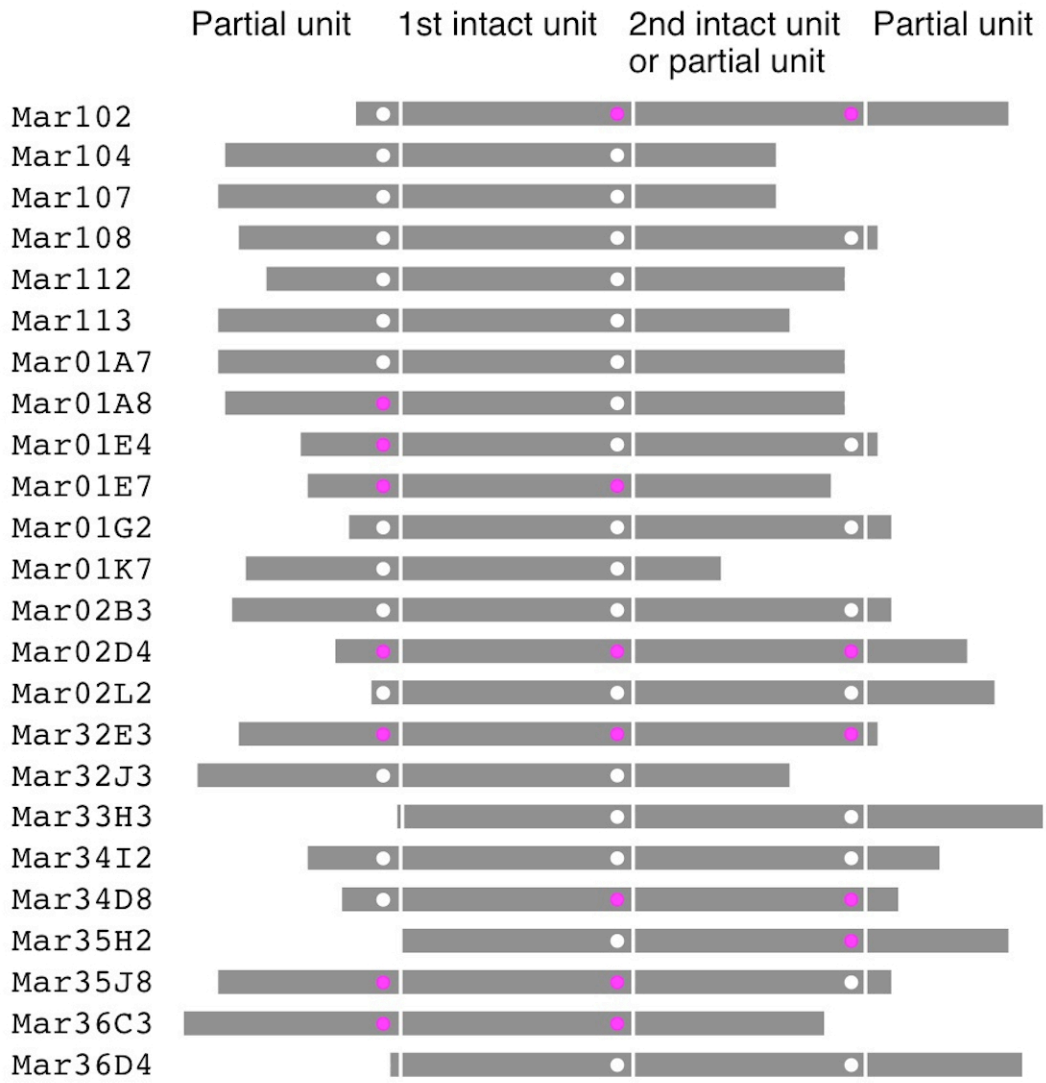

Figure S3. Distribution of CENP-B boxes among repeat units contained in sequence reads. The names of the 24 fosmid clones are listed on the left of the alignment. Partial or intact repeat units contained in their sequence reads are schematically shown. The circles located near the right ends of the repeat units indicate sites for CENP-B box. Magenta-coloured and white circles indicate the presence and absence of CENP-B boxes, respectively.

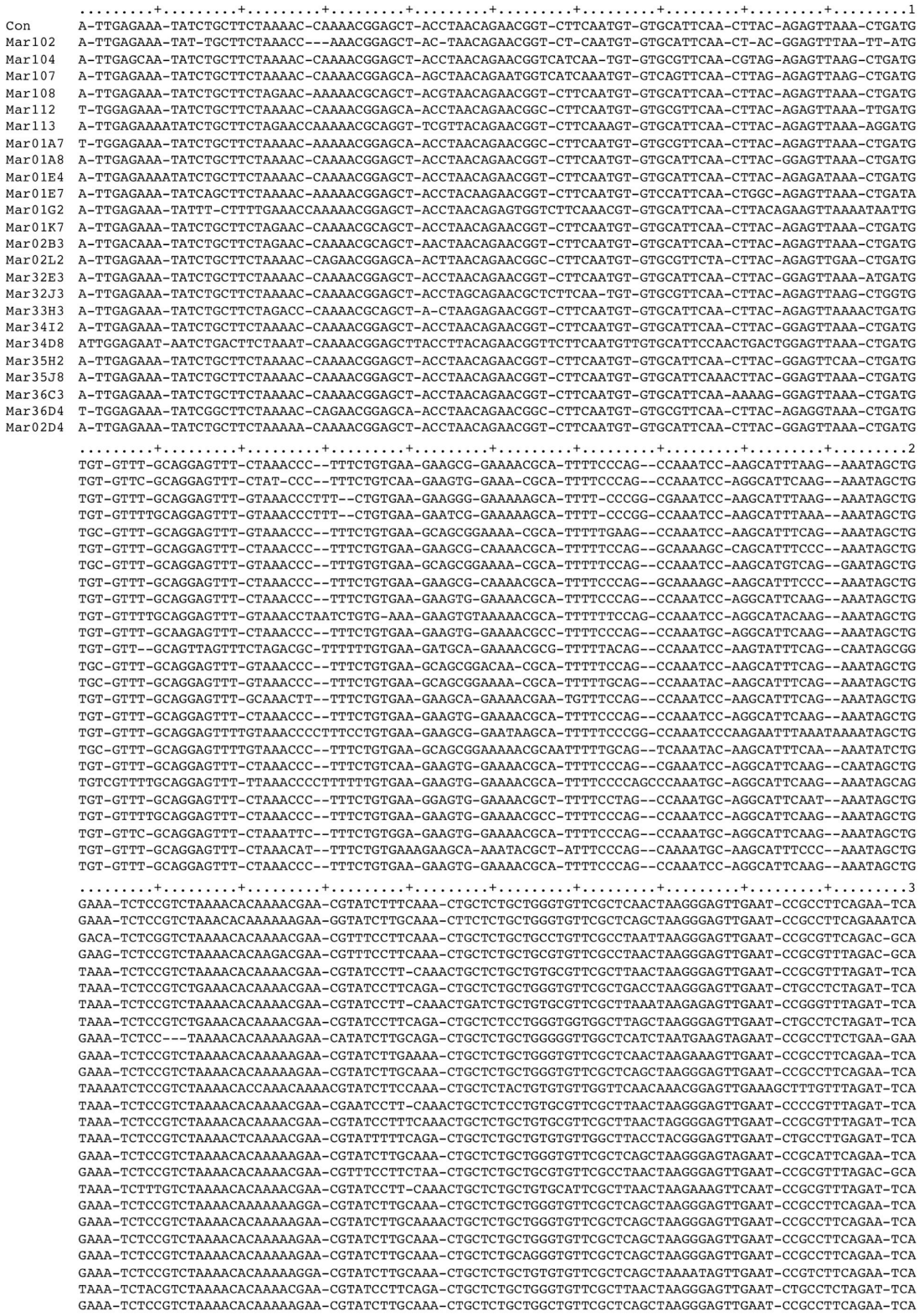

Figure S4. Alignment of entire sequences of first intact repeat units. The names of the fosmid clones are listed on the left of the alignment. For additional information see the legend to Figure 1.

Figure S4 (continued)