Supplemental Materials for

Birth, evolution and transmission of satellite-free mammalian centromeric domains

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SUPPLEMENTAL METHODS

Cell lines

Primary fibroblast cell lines from HorseS and DonkeyA were established from the skin of slaughtered animals. Fibroblasts from DonkeyB, HorseA and HorseC and Hinny were established from skin biopsies of adult animals from Cornell University. HorseD fibroblasts were obtained from testicular tissue of a freshly castrated animal from Cornell. MuleA, MuleB and MuleC cell lines were derived from three mule conceptuses from normal pregnancies recovered on days 32-34 after ovulation via uterine lavage, as described (Adams and Antczak 2001). Conceptuses were dissected under a microscope into discrete tissues, including the tail/hind quarter region which is comprised of primarily fibroblasts at this stage of development. The tail region was minced, spun down, washed in 1x Phosphate Buffered Saline (PBS) and resuspended in DMEM (Invitrogen) plus 10% FBS for culture.

Immortalization of the MuleA fibroblast cell line was carried out as described previously with minor modifications (Vidale et al. 2012). Briefly, in a plasmid containing the neomycin resistance gene and the human telomerase reverse transcriptase (pCi-hTERT) we inserted the gene for the human telomerase RNA component (hTERC). The hTERT-TERC plasmid was then used to transfect primary fibroblasts from MuleA with the transfecting agent FuGENE 6 (Roche). Forty-eight hours after transfection, 0.4 mg/ml G418 sulphate (Invitrogen) was added. After about three weeks resistant colonies started to grow. Resistant clones were then pooled and cultured to obtain an immortalized cell population.

Primary fibroblasts were cultured in high-glucose DMEM medium (EuroClone) medium supplemented with: 20% fetal calf serum (EuroClone), 2x NEAA (non essential amino acids, EuroClone), 2mM L-glutamine (SIGMA), 1x penicillin/streptomycin (SIGMA).

MuleA immortalized fibroblasts were grown in the same medium of primary fibroblasts supplemented with G418. Immortalized cells were seeded at low density in order to obtain several discrete clones that were isolated and propagated independently. All cells were maintained in a humidified atmosphere of 5% CO2 at 37°C.

Sequencing of ChIP enriched DNA

Three replicate experiments were performed for the DonkeyB, MuleA, MuleB and MuleC cell lines. Briefly, two independent chromatin crosslinking and immuno-precipitation experiments were performed. From the first immuno-precipitation two separate Illumina libraries were independently prepared and paired-end sequenced; the corresponding input sample was sequenced just once. The two ChIP-seq datasets deriving from the first immuno-precipitation were named “replicate 1” and “replicate 2”; the corresponding input dataset was named “replicate 1-2”. The second immuno-precipitation was used to prepare an independent library and paired-end sequenced, in parallel with its corresponding input. The resulting datasets were named “replicate 3”. A single ChIP-seq assay was carried out for the following cell lines: DonkeyA, HorseS, HorseA, HorseB, HorseC, HorseD and Hinny. All sequencing experiments were performed through an Illumina HiSeq2000 or HiSeq2500 platform by IGA Technology Services (Udine, Italy). The length and number of reads obtained in each dataset are reported in Supplemental Table S1.

Cytogenetic analysis

FISH experiments on horse and donkey metaphase spreads were carried out with a panel of BAC clones from the horse library CHORI-241 (Supplemental Table S2). The equine BAC clones were labeled by nick translation with Alexa Fluor® 488-5-d-UTP (Invitrogen) or Cy3-dUTP (Enzo Life Sciences) and hybridized to horse and donkey metaphase spreads. Chromosomes were counterstained with DAPI. Digital grey-scale

images for Cy3, Alexa 488 and DAPI fluorescence signals were acquired with a fluorescence microscope (Zeiss Axioscope.A1) equipped with a cooled CCD camera (Photometrics). Pseudo-colouring and merging were performed using the IpLab software. Chromosomes were identified by computer generated reverse DAPI banding according to the standard karyotypes.

Assembly of centromeric regions, sequence analysis and construction of the chimeric reference genomes

The *de novo* assembly of the donkey centromeric regions was performed using an iterative chromosome walking approach based on ChIP-seq reads. The definition of centromeric sequences was completed with PCR amplification and Sanger sequencing of selected regions, which enabled resolution of gaps, identification of rearrangements and confirmation of the correctness of our assembly. The length of the resulting contigs is reported in Supplemental Table S3.

The analysis of DNA sequence elements in the DonkeyA centromeric regions and of the orthologous horse sequence (EquCab2) was carried out with RepeatMasker (http://www.repeatmasker.org). The results of the analysis on the donkey centromeres were compared with average genome-wide values (Huang et al. 2015). Statistical significance was evaluated with a one-sample *t-test*.

To construct the chimeric EquCabAsiA and EquCabAsiB references, we used BLAT (Kent 2002) to identify the regions of the horse EquCab2 genome that are orthologous to the donkey centromeric contigs; these regions were removed and substituted with the newly-assembled donkey centromeric sequence obtained from

DonkeyA (EquCabAsiA) or DonkeyB (EquCabAsiB). Supplemental Table S3 reports the coordinates of the regions which were removed from EquCab2.

Bioinformatic analysis of ChIP-seq data

Reads were aligned to the horse reference genome (EquCab2.0, 2007 release) or to the EquCabAsiA or EquCabAsiB references with Bowtie 2.0 (Langmead and Salzberg 2012). using default parameters. We were interested in identifying satellite-free centromeric domains on unique sequences which are well assembled in EquCab2.0. Peak calling was performed with the software MACS 2.0.10 (Zhang et al. 2008). We did not include in our analysis reads not assigned to a particular chromosome (chrUn). In donkey A, the percentage of ChIP-seq reads mapping on chrUn was 10.4 with the anti-CENPA antibody and 5.9 with the CREST serum. Supplemental Table S1 reports sequencing and alignment statistics of all datasets. ChIP-seq data were normalized with the deepTools package using a subtractive method (Ramírez et al. 2014). Briefly, for both input and ChIP datasets, the number of reads mapping in each 1kb-wide genomic window was divided by the total number of mapped reads (expressed in millions), thus obtaining a RPKM value (Reads Per Kilobase per Million mapped reads). Then, for each genomic window, the input signal was subtracted from the ChIP signal, resulting in the normalized read count. All ChIP-seq enrichment plots were obtained with the R software package Sushi (Phanstiel et al. 2014). Input reads of HorseS, DonkeyA, DonkeyB replicate 1, MuleA replicate1 were used to detect sequence amplification (Fig. 2): HorseS, DonkeyA and MuleA datasets were downsampled to a total read number comparable to DonkeyB using Picard version 1.140 (http://broadinstitute.github.io/picard). All datasets were then mapped on EquCab2.0 reference genome and plotted with IGV (Robinson et al. 2011)

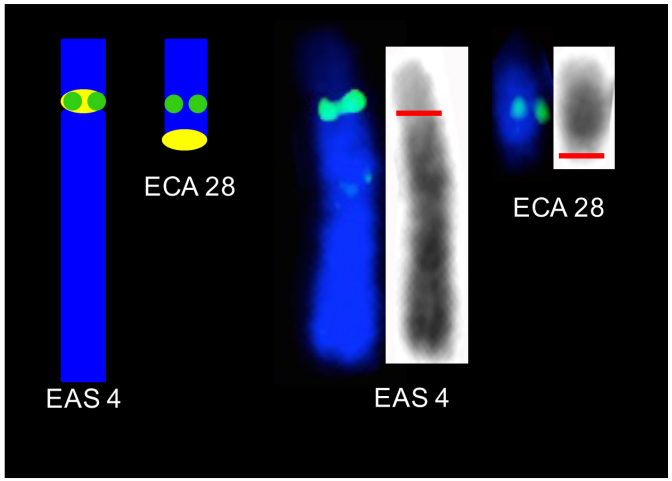
SNV analysis

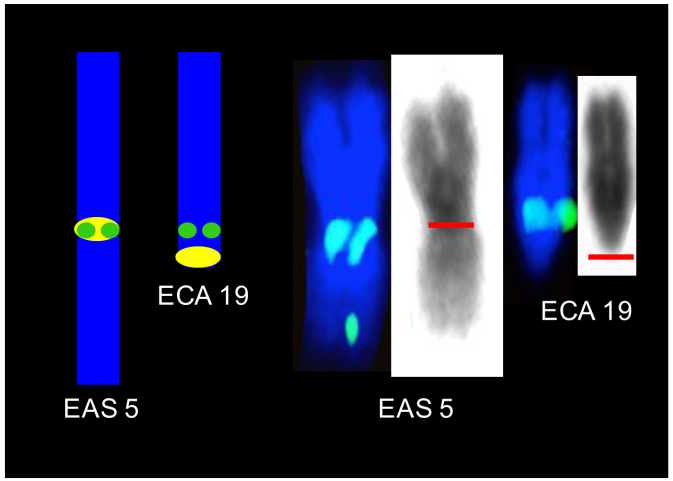
To identify single nucleotide variation (SNV) in the DonkeyA centromeric regions, we employed the IGV software (Robinson et al. 2011) with the EquCabAsiA genome as reference. We analyzed the BAM file resulting from the mapping of input reads from DonkeyA and searched for SNVs using the following filter parameters: remove duplicated reads, vendor failed reads and secondary alignments. We set the coverage allele-fraction threshold at 0.4 to single out nucleotides in which two variants were present. Minimum number of reads required for the analysis was 4. We used only SNVs present within single copy sequences. We then loaded the ChIP-seq BAM file to detect variants present in the centromeric domains: if two variants were present (least frequent variant equal or more than 30%) both homologs were scored as having a CENPA binding domain in that region; if only one variant was present (least frequent variant less than 30%), only one homolog was scored as having a CENPA binding domain in that position.

Figure S1: FISH mapping of donkey satellite-free centromeric domains

The localization of *E. asinus* CENP-A binding domains on primary constrictions was confirmed using BAC probes covering the genomic regions identified by ChIP-seq. The chromosomes not included in Figure 1 C are shown here. On the left of each panel a sketch of the donkey chromosomes and of their horse ortholog is shown: orthologies and primary constriction positions (yellow ovals) were deduced from Yang et al 2004 and Musilova et al 2013; green dots correspond to the position of the BAC-FISH signals observed in the present work. On the right of each panel, metaphase chromosomes are shown with FISH signals in green and the primary constriction marked by a red line on the reverse DAPI images (grey). According to Musilova et al. 2013, the position of the primary constriction of EAS12 coincides with that of the orthologous ECA9. However, our FISH data show that, on ECA9, the BAC-FISH signal is on the proximal region of the short arm, adjacent to the primary constriction, thus the position of the primary constriction of ECA9 has been sketched accordingly. Therefore, also this centromere is repositioned, confirming the ChIP-seq results.

Figure S1


ECA 28
ECA 28
EAS 4
EAS 4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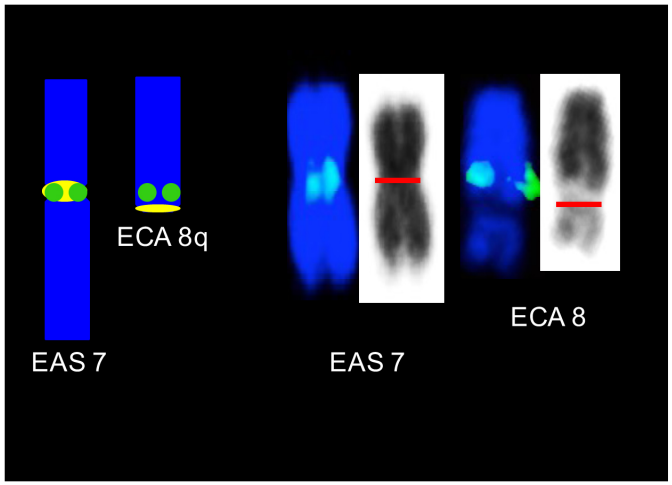
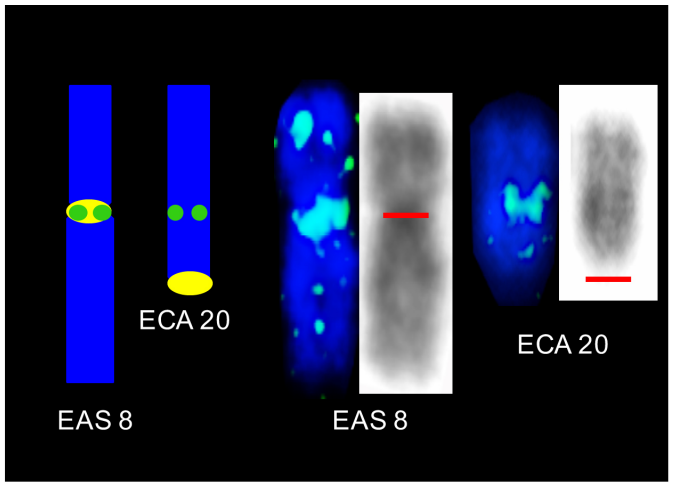
ECA 19
EAS 5
EAS 5


ECA 8q
ECA 8
EAS 7
EAS 7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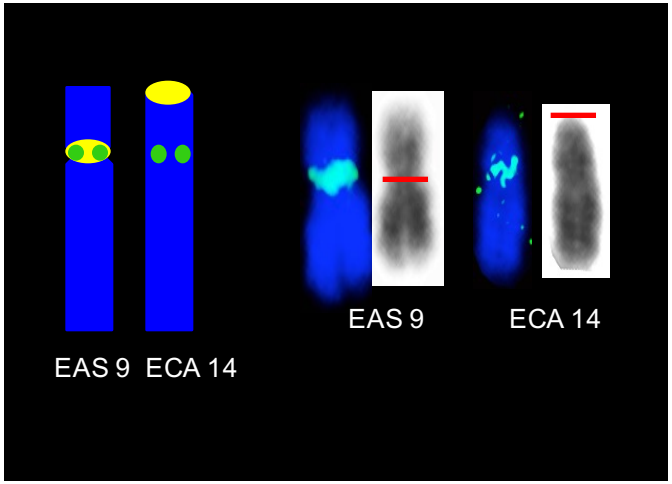
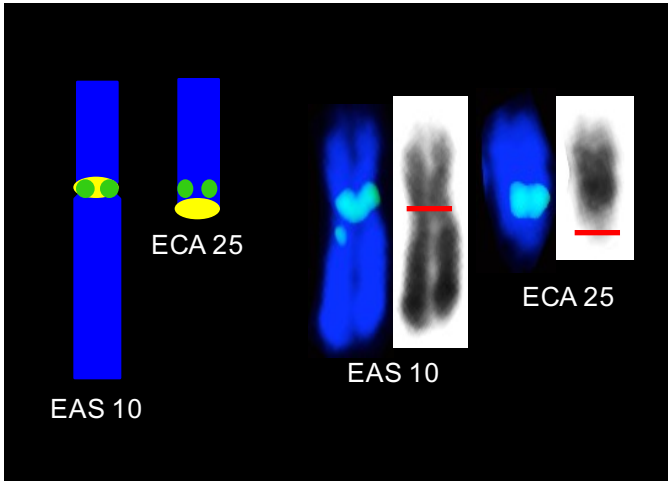
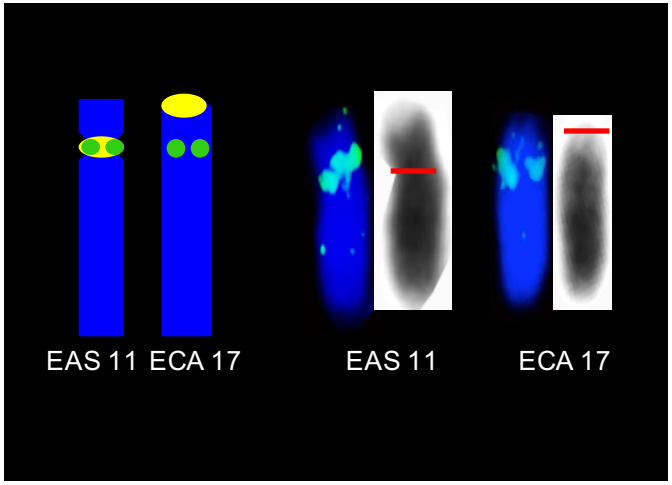
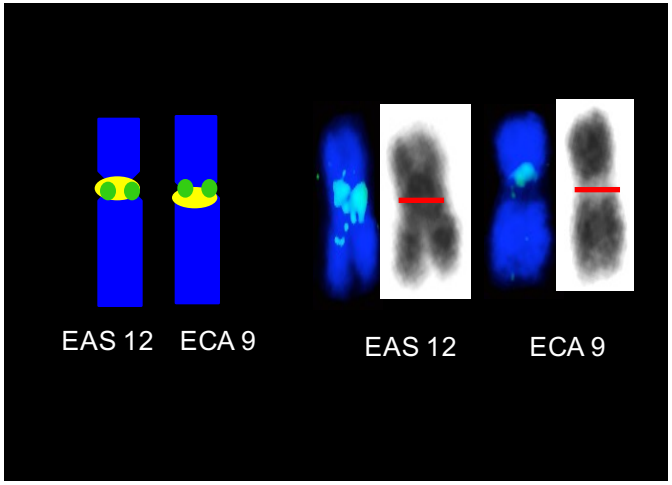
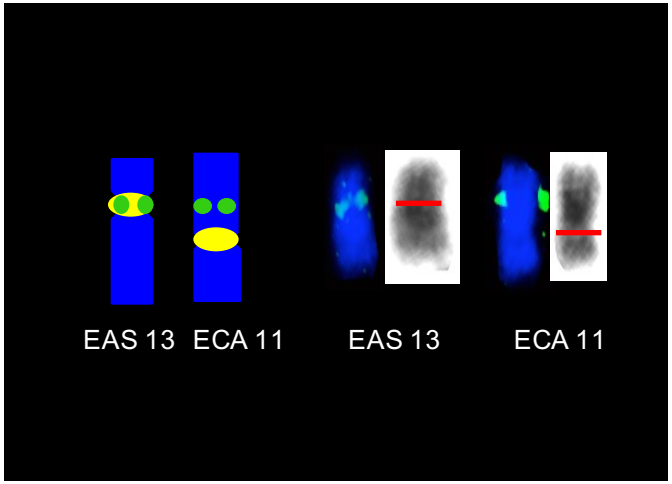
ECA 20
EAS 8
EAS 8


EAS 9
ECA 14
EAS 9
ECA 14


ECA 25
EAS 10
EAS 10
ECA 25


EAS 11
ECA 17
EAS 11
ECA 17


EAS 12
ECA 9
EAS 12
ECA 9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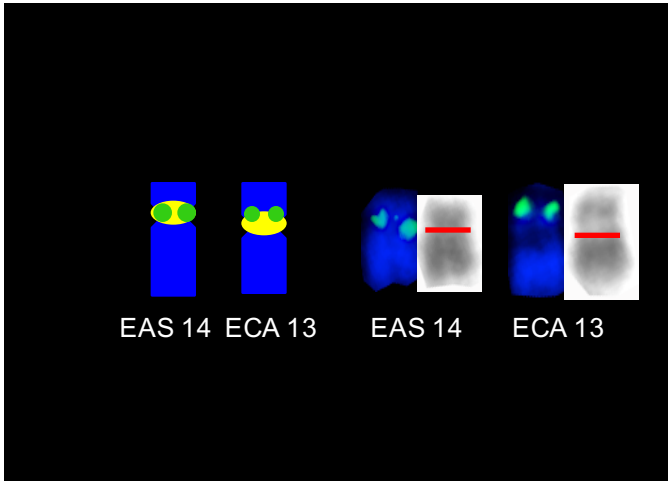
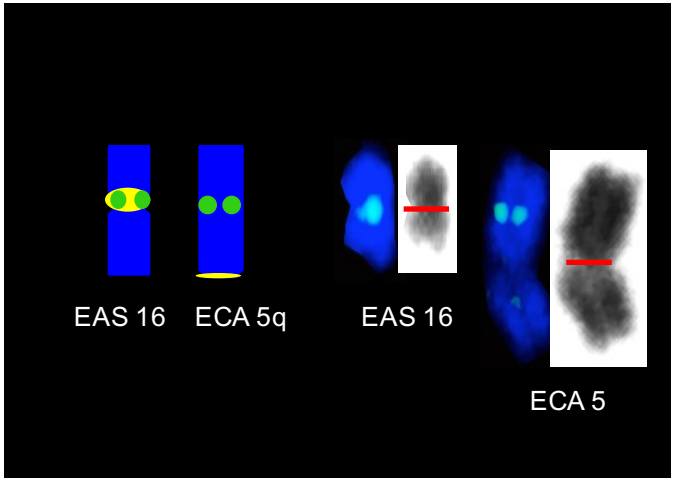
ECA 11
EAS 13
ECA 11


EAS 14
ECA 13
EAS 14
ECA 13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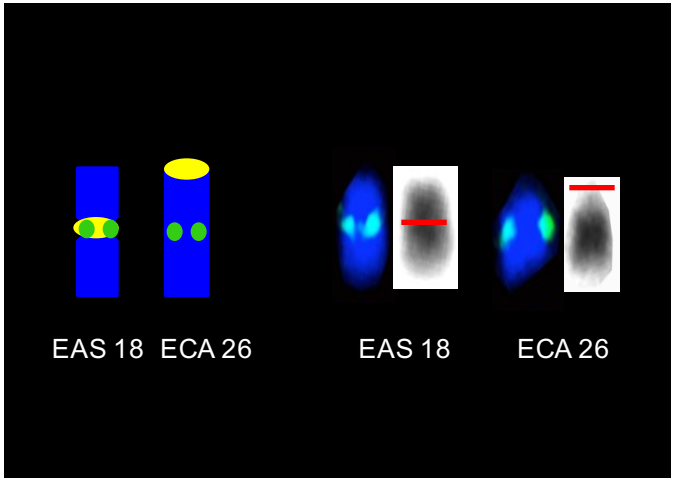
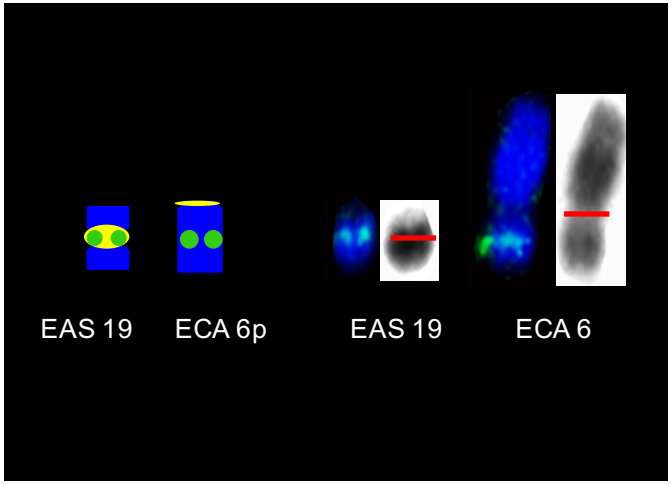
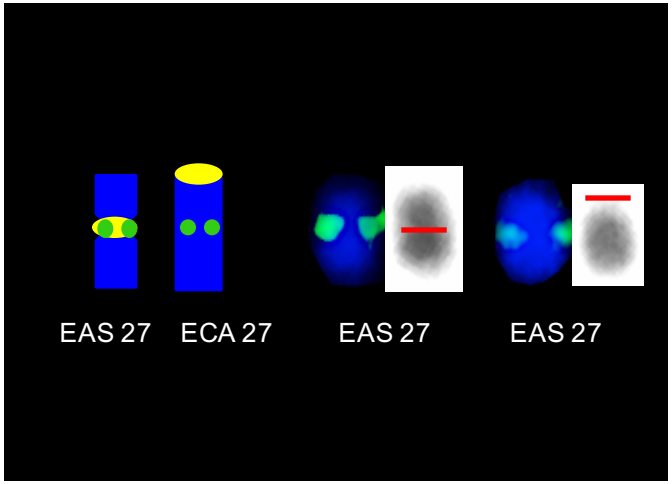
ECA 5q
EAS 16
ECA 5


EAS 18
ECA 26
EAS 18
ECA 26


EAS 19
ECA 6p
EAS 19
ECA 6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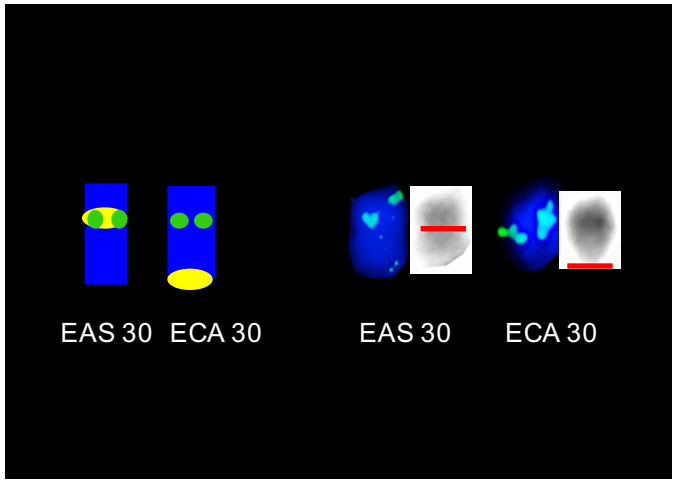
ECA 27
EAS 27
EAS 27


EAS 30
ECA 30
EAS 30
ECA 30

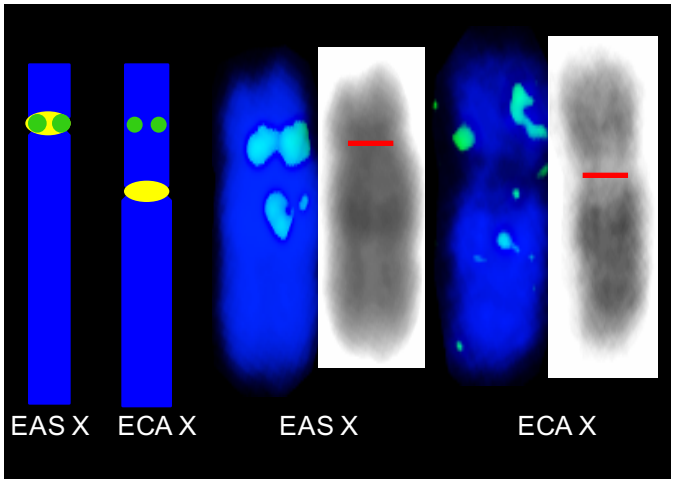
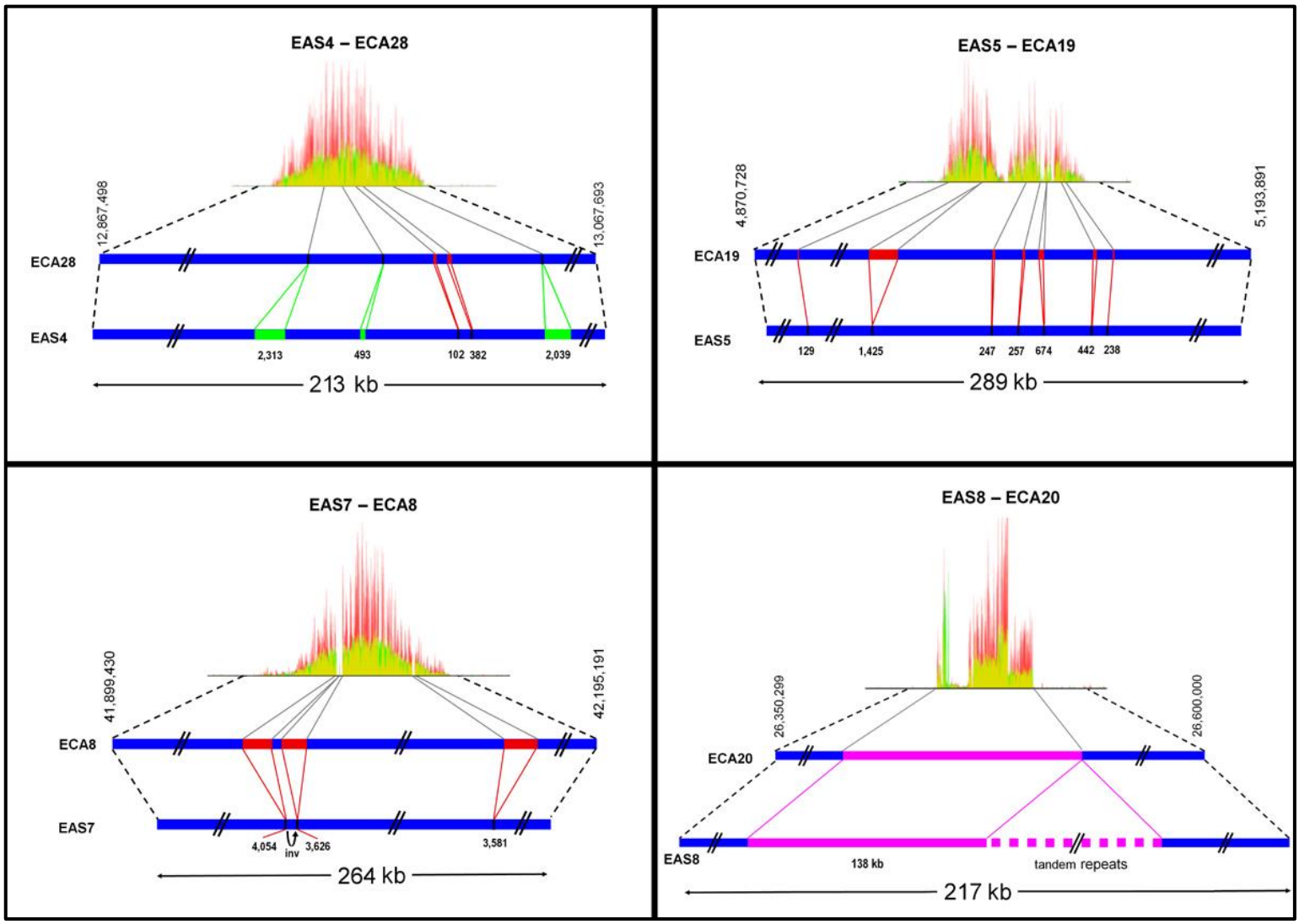
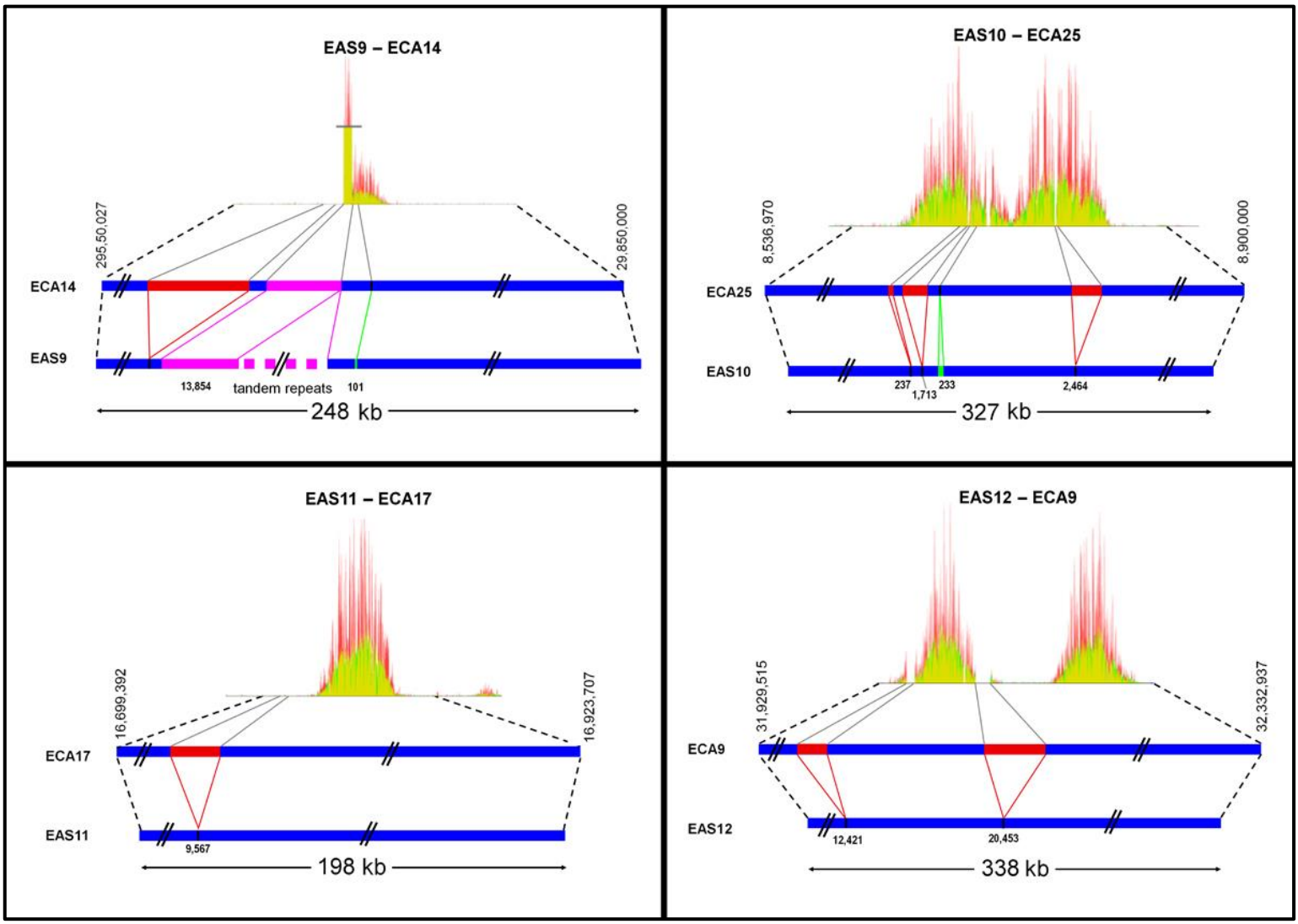

EAS X
ECA X
EAS X
ECA X

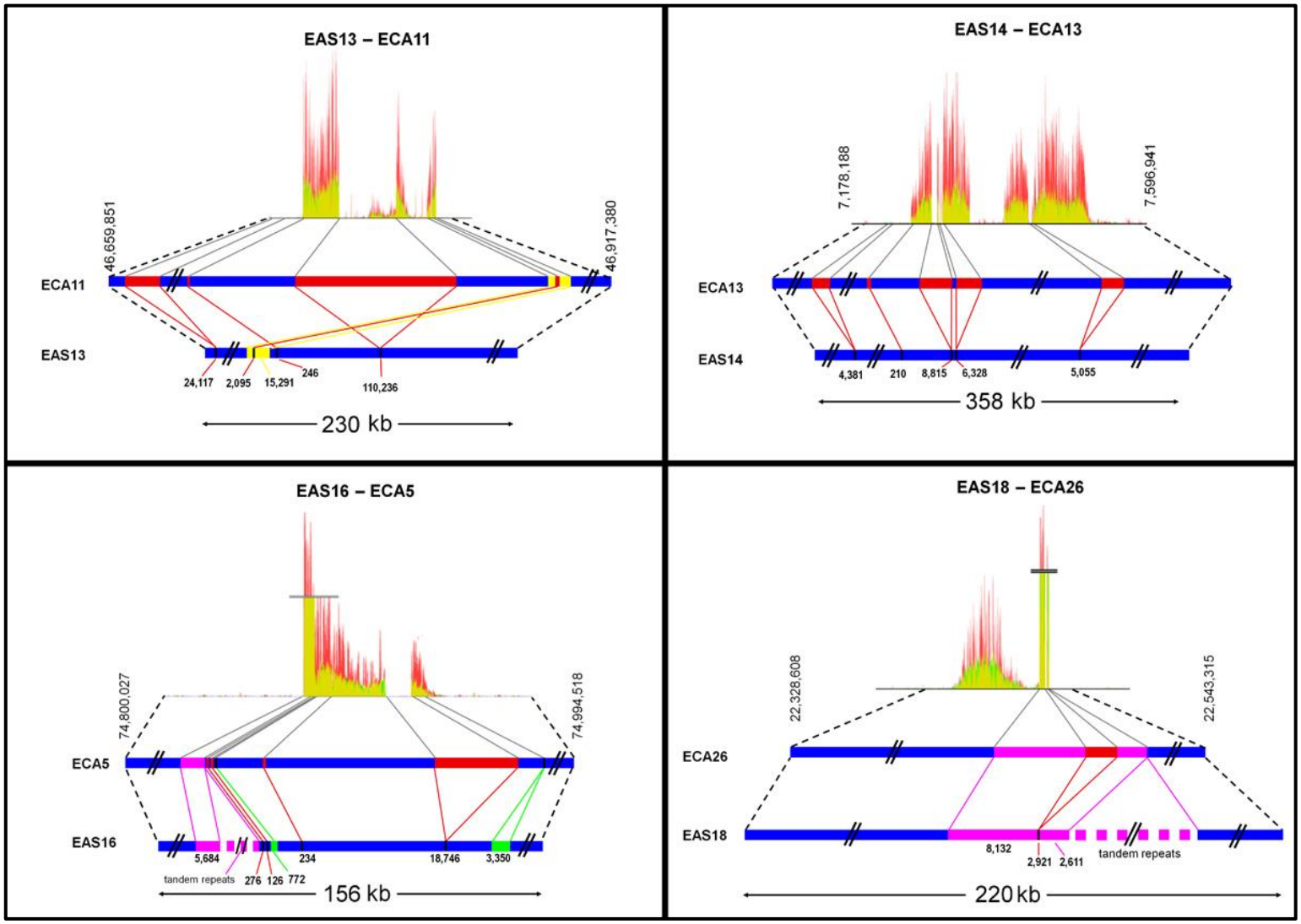
Figure S2: Sequence rearrangements at the 16 donkey satellite-less centromeric domains in comparison with their horse orthologous regions

Each panel refers to a single satellite-less centromere. In each panel, the ChIP-seq profile of the CENP-A binding domain, as reported in Figure 1B, is shown. The top blue line represents the horse reference genomic sequence (EquCab2.0) with coordinates indicated at the borders. The bottom blue line represents the de novo assembled sequence from DonkeyA. Black double slashes indicate DNA fragments whose sequence is known but is not included in the figure. Only sequence rearrangements of more than 100 bp are reported. Sequence amplifications in the donkey with respect to the horse are reported in pink. Insertion and deletion in the donkey compared to the horse are indicated in green and in red, respectively, with their size in base pairs reported below. The black arrow at donkey chromosome 7 represents a sequence inversion (inv). At donkey chromosome 13 a translocation was found which is marked in yellow.

Figure S2


EAS4 – ECA28
12,867,498
13,067,693
ECA28
EAS4
2,313
493
102 382
2,039
213 kb
EAS5 – ECA19
4,870,728
5,193,891
ECA19
EAS5
129
1,425
247 257 674
442 238
289 kb
EAS7 – ECA8
41,899,430
42,195,191
ECA8
EAS7
4,054
inv
3,626
3,581
264 kb
EAS8 – ECA20
26,350,299
26,600,000
ECA20
EAS8
138 kb
tandem repeats
217 kb


EAS9 – ECA14
295,50,027
29,850,000
ECA14
EAS9
13,854
tandem repeats
101
248 kb
EAS10 – ECA25
8,536,970
8,900,000
ECA25
EAS10
237
1,713
233
2,464
327 kb
EAS11 – ECA17
16,699,392
16,923,707
ECA17
EAS11
9,567
198 kb
EAS12 – ECA9
31,929,515
32,332,937
ECA9
EAS12
12,421
20,453
338 kb


EAS13 – ECA11
46,659,851
46,917,380
ECA11
EAS13
24,117
2,095
15,291
246
110,236
230 kb
EAS14 – ECA13
7,178,188
7,596,941
ECA13
EAS14
4,381
210
8,815
6,328
5,055
358 kb
EAS16 – ECA5
74,800,027
74,994,518
ECA5
EAS16
5,684
tandem repeats
276
126
772
234
18,746
3,350
156 kb
EAS18 – ECA26
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22,543,315
ECA26
EAS18
8,132
2,921
2,611
tandem repeats
220 kb

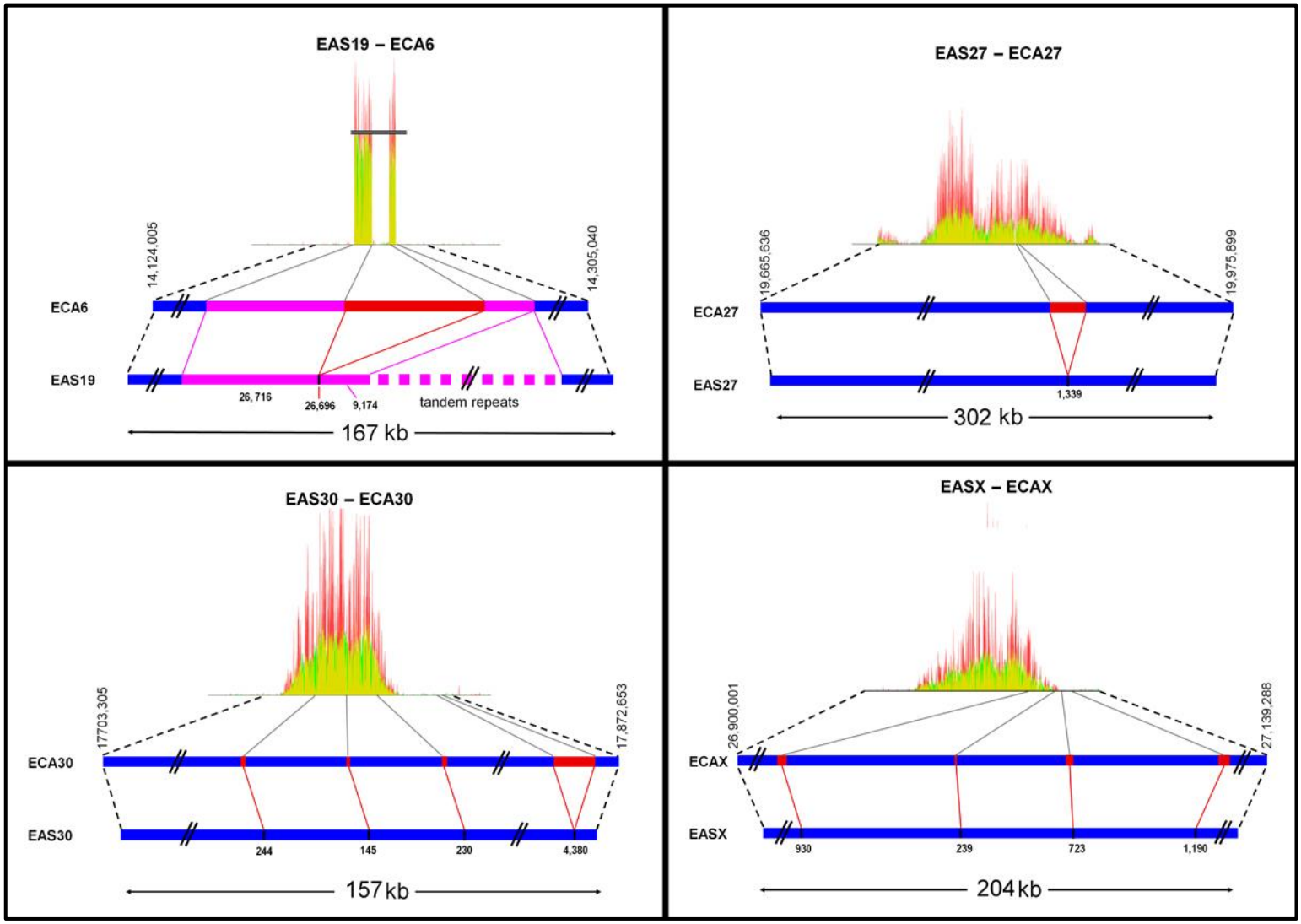

EAS19 – ECA6
14,124,005
14,305,040
ECA6
EAS19
26, 716
26,696
9,174
tandem repeats
167 kb
EAS27 – ECA27
19,665,636
19,975,899
ECA27
EAS27
1,339
302 kb
EAS30 – ECA30
17703,305
17,872,653
ECA30
EAS30
244
145
230
4,380
157 kb
EASX – ECAX
26,900,001
27,139,288
ECAX
EASX
930
239
723
1,190
204 kb

Figure S3: Mapping of ChIP-seq reads on the EquCabAsiA chimeric reference genome

The ChIP-seq reads of DonkeyA, previously aligned with the horse reference genome (Figure 1B), are here mapped on the EquCabAsiA chimeric reference genome.

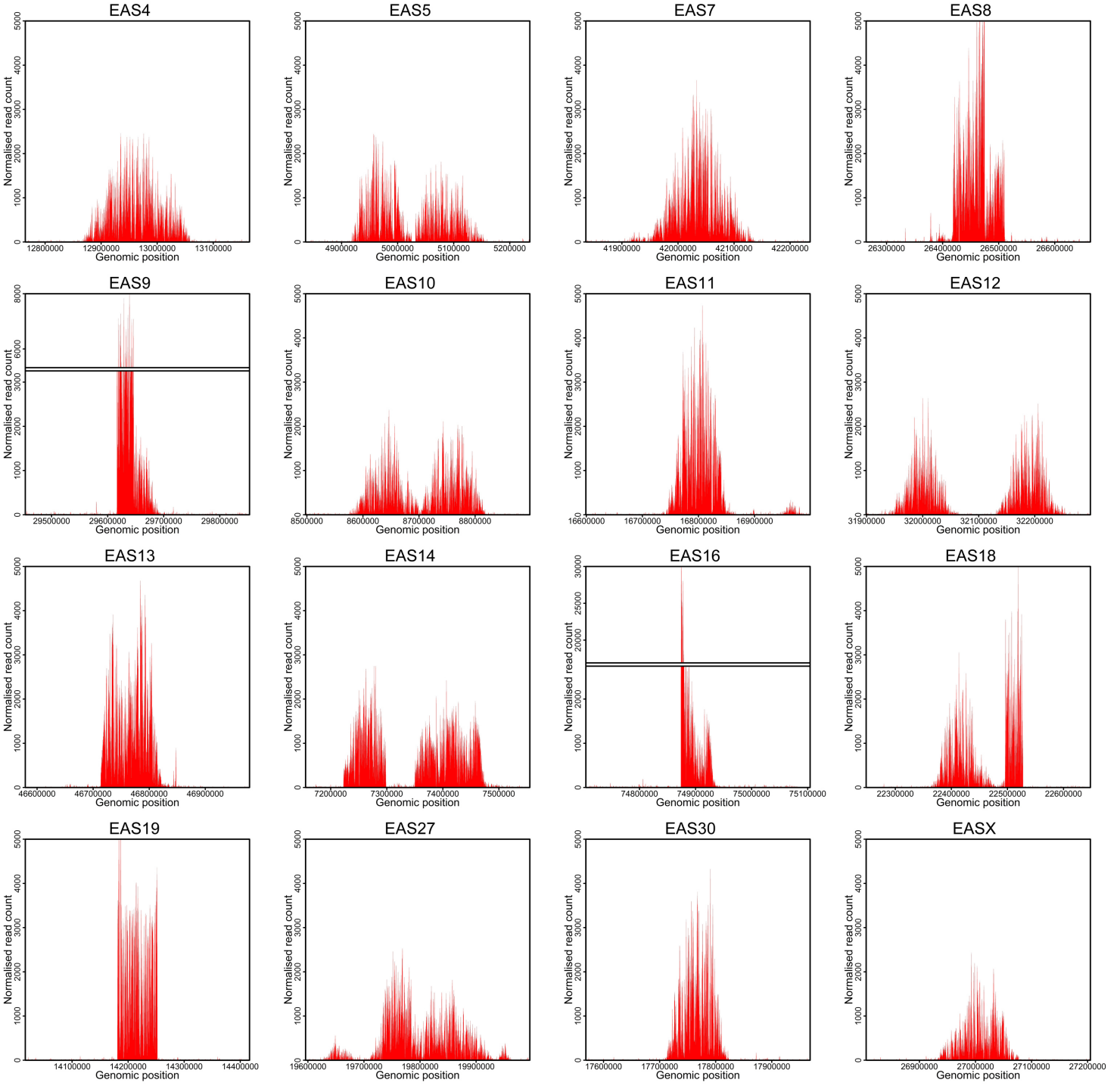

EAS4
EAS5
EAS7
EAS8
EAS9
EAS10
EAS11
EAS12
EAS13
EAS14
EAS16
EAS18
EAS19
EAS27
EAS30
EASX
Normalised read count
Genomic position

Figure S4: DNA sequence amplification at the centromeres of *E. asinus* chromosomes 16 and 8

A: quantitative PCR performed on DNA from two horses (HorseS and HorseT), two donkeys (DonkeyA and DonkeyB) and one mule (MuleA). Amplification levels were calculated using a fragment from the horse TERC gene as single-copy reference and assigning the value of 1 to the amplification level of HorseS. The centromere of chromosome 16 was analyzed with three primer pairs (red, green and blue bars), while the centromere of chromosome 8 was analyzed with 4 primer pairs (red, green, blue and grey). **B**: Profile of input reads from one horse (HorseS), two donkeys (DonkeyA and DonkeyB) and one mule (MuleA) aligned on the horse reference genome. The genomic regions shown are: 74,821,645-74,953,046 for chromosome 16 and 26,330,485-26,592,393 for chromosome 8. Peaks represent regions amplified in the donkey genome compared to the horse genome. Colored triangles indicate the location of the fragments amplified in the quantitative PCR assay (A).

Figure S4

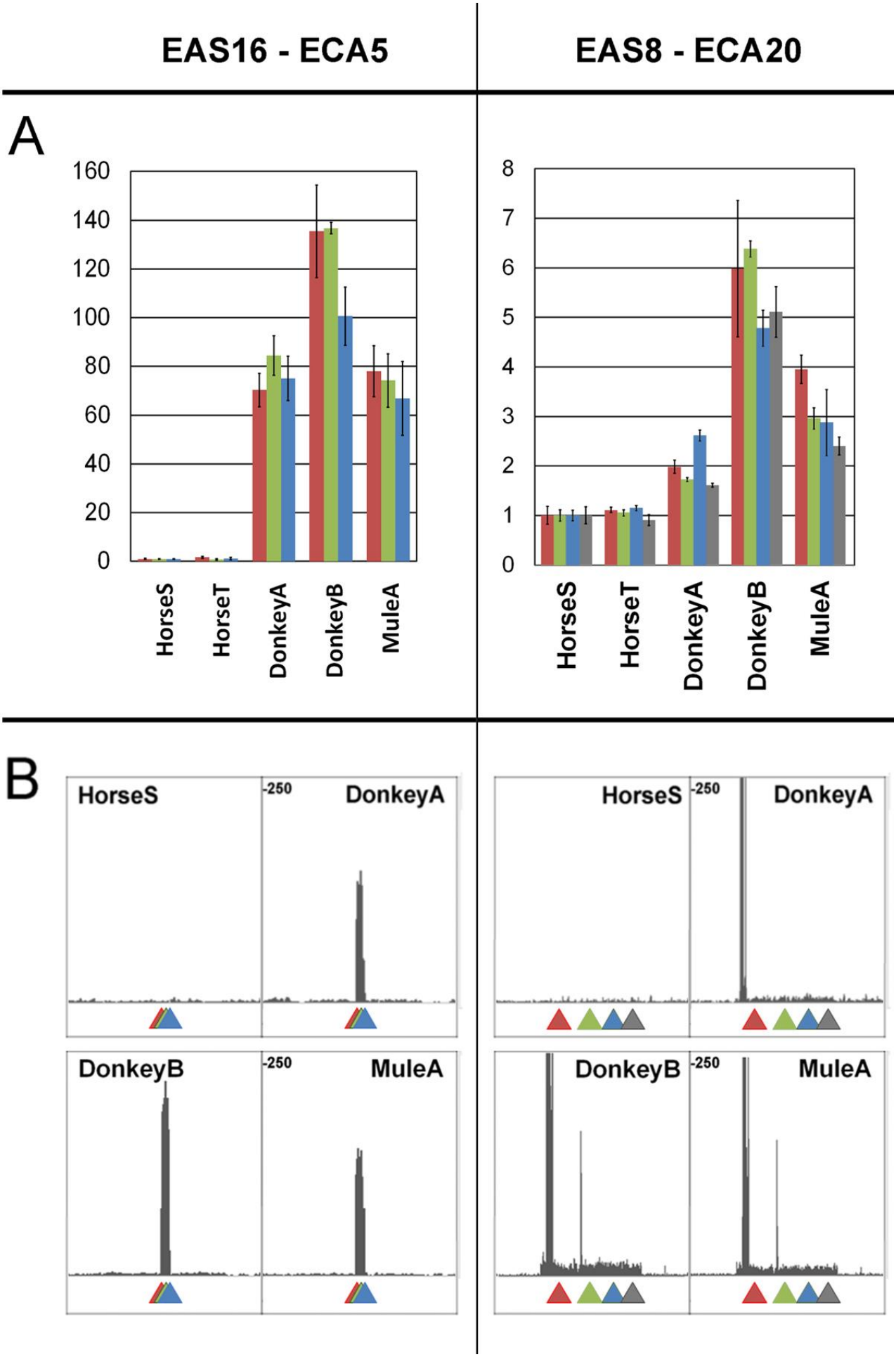

EAS16 - ECA5
EAS8 - ECA20
A
160
140
120
100
80
60
40
20
0
8
7
6
5
4
3
2
1
0
HorseS
HorseT
DonkeyA
DonkeyB
MuleA
B
HorseS
-250
DonkeyA
DonkeyB
-250
MuleA

Figure S5: Sequence analysis of the 16 satellite-less donkey centromeric domains

The satellite-free donkey genomic sequences assembled here (black bars) were pooled and the GC, SINEs, LINEs, LTRs and DNA elements content calculated. The values obtained were compared with those calculated from the pooled horse orthologous regions (dark grey bars) and from donkey genome wide average [36] (light grey bars). Asterisks indicate statistically significant differences, with a p-value < 0.001 in the one-sample t-test.

Figure S5

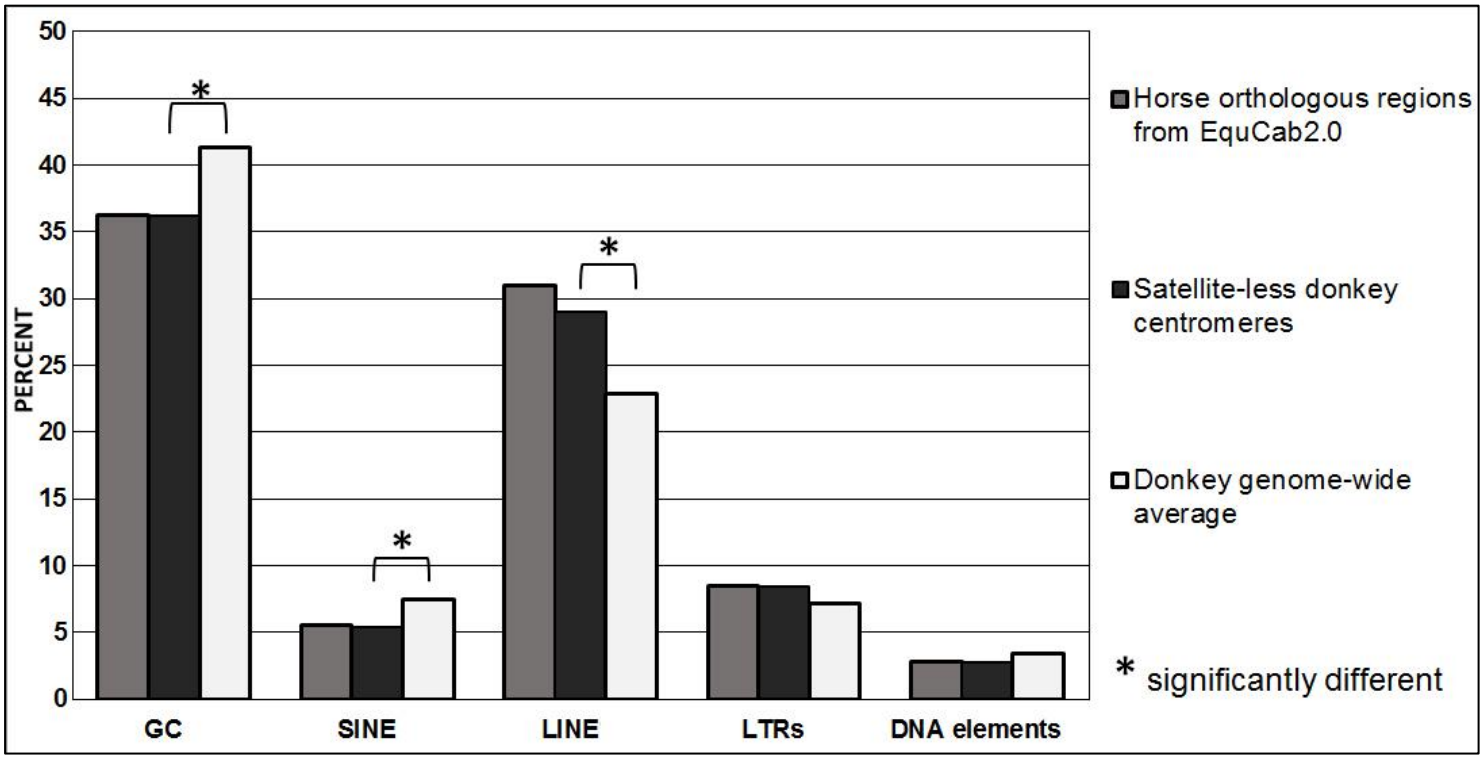

50
45
40
35
30
25
20
15
10
5
0
PERCENT
*
*
*
GC
SINE
LINE
LTRs
DNA elements
Horse orthologous regions from EquCab2.0
Satellite-less donkey centromeres
Donkey genome-wide average
* significantly different

Figure S6: Centromere sliding at DonkeyA and DonkeyB centromeric domains

The position of CENP-A binding domains was compared between DonkeyA and DonkeyB. Reads from EAS4, EAS5, EAS7, EAS10, EAS12 and EAS18 were mapped on the EquCab2.0 horse reference genome. The Y axis reports the normalized read count, obtained by subtracting the input, while the x axis reports the genomic coordinates. The top and the bottom of each panel report CENP-A binding domains of DonkeA (red) and DonkeyB (black), respectively.

Figure S6

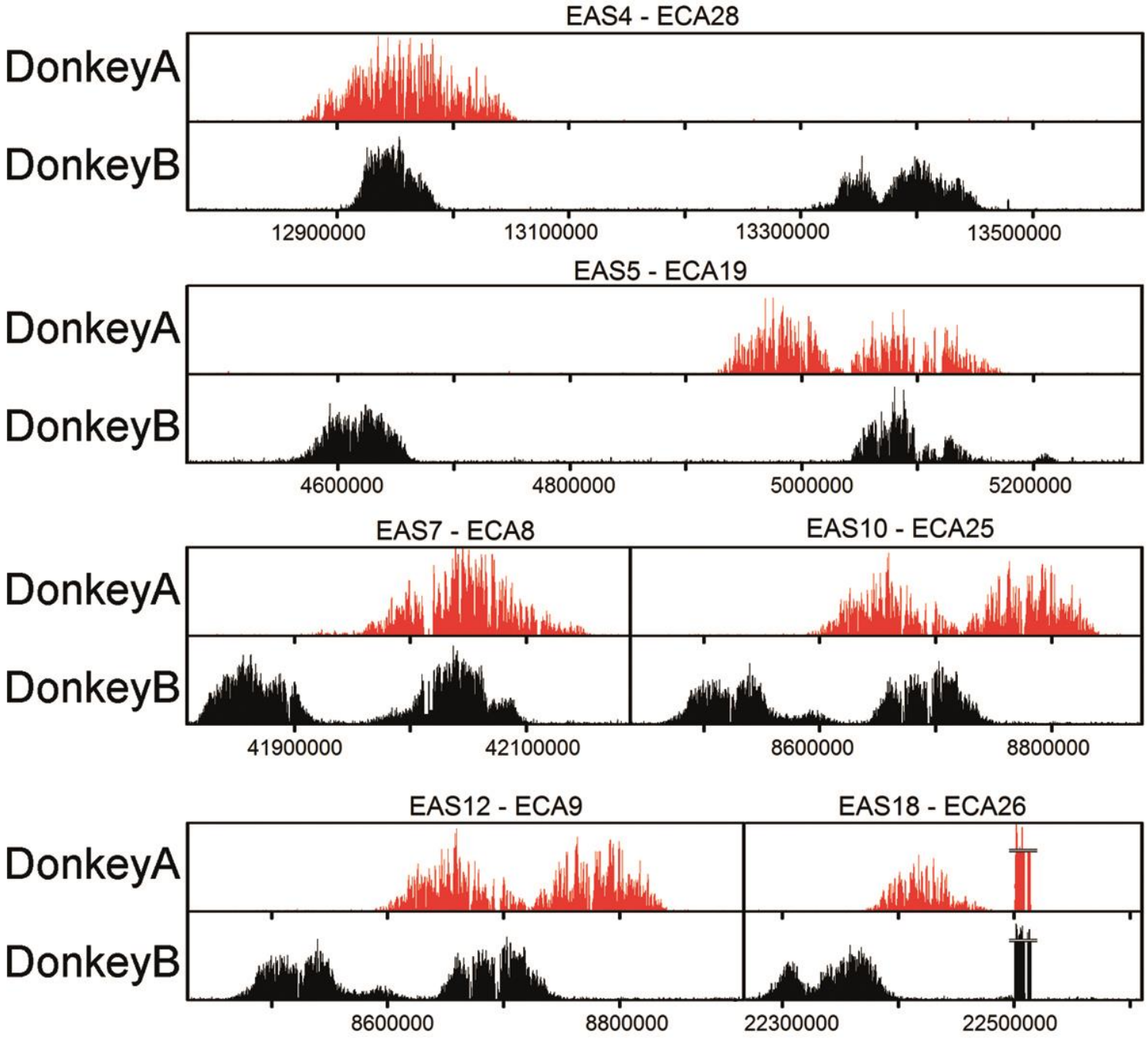
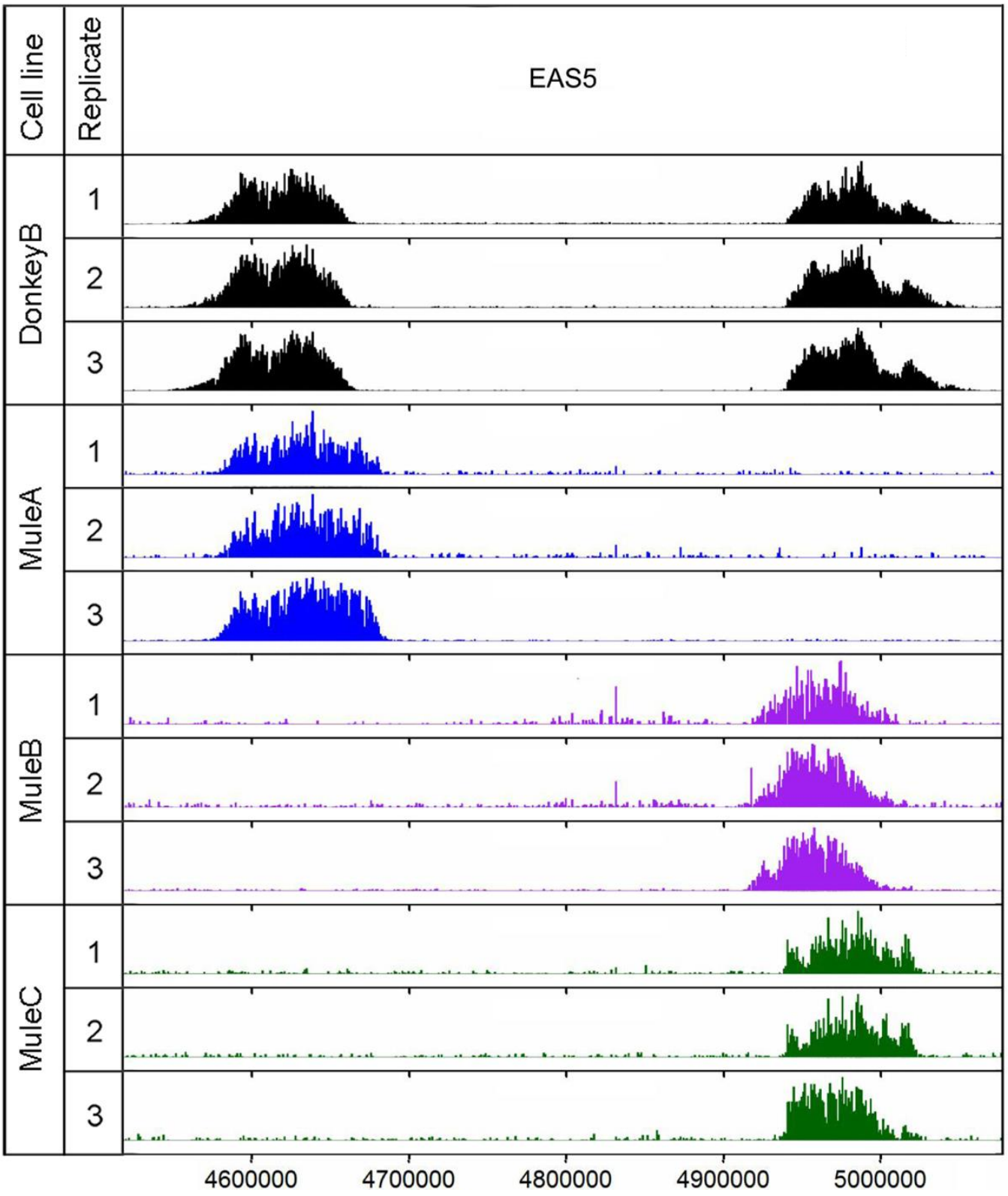
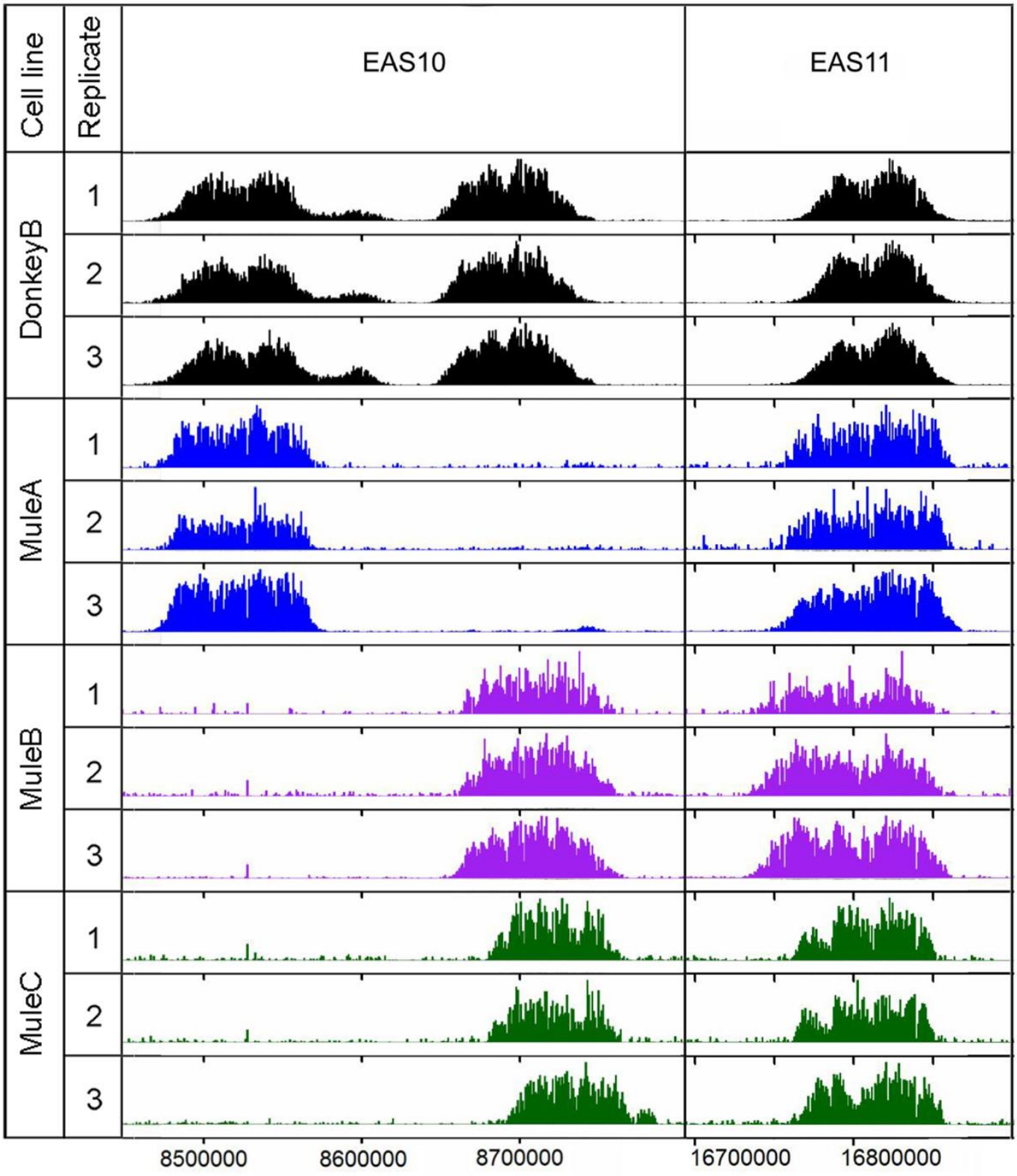

EAS4 - ECA28
DonkeyA
DonkeyB
12900000
13100000
13300000
13500000
EAS5 - ECA19
DonkeyA
DonkeyB
4600000
4800000
5000000
5200000
EAS7 - ECA8
EAS10 - ECA25
DonkeyA
DonkeyB
41900000
42100000
8600000
8800000
EAS12 - ECA9
EAS18 - ECA26
DonkeyA
DonkeyB
8600000
8800000
22300000
22500000

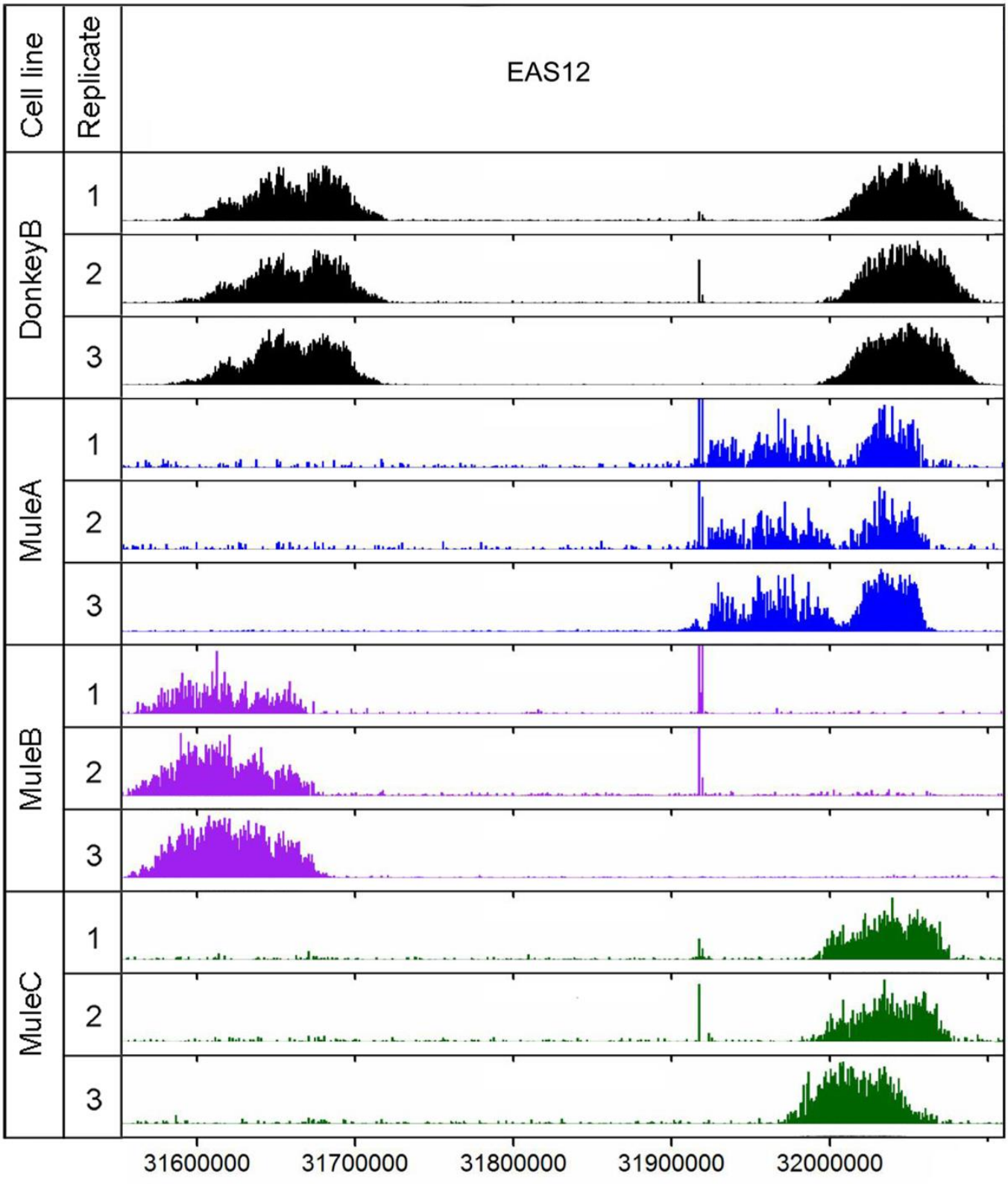
Figure S7: Transmission of satellite-less centromeric domains in hybrid families

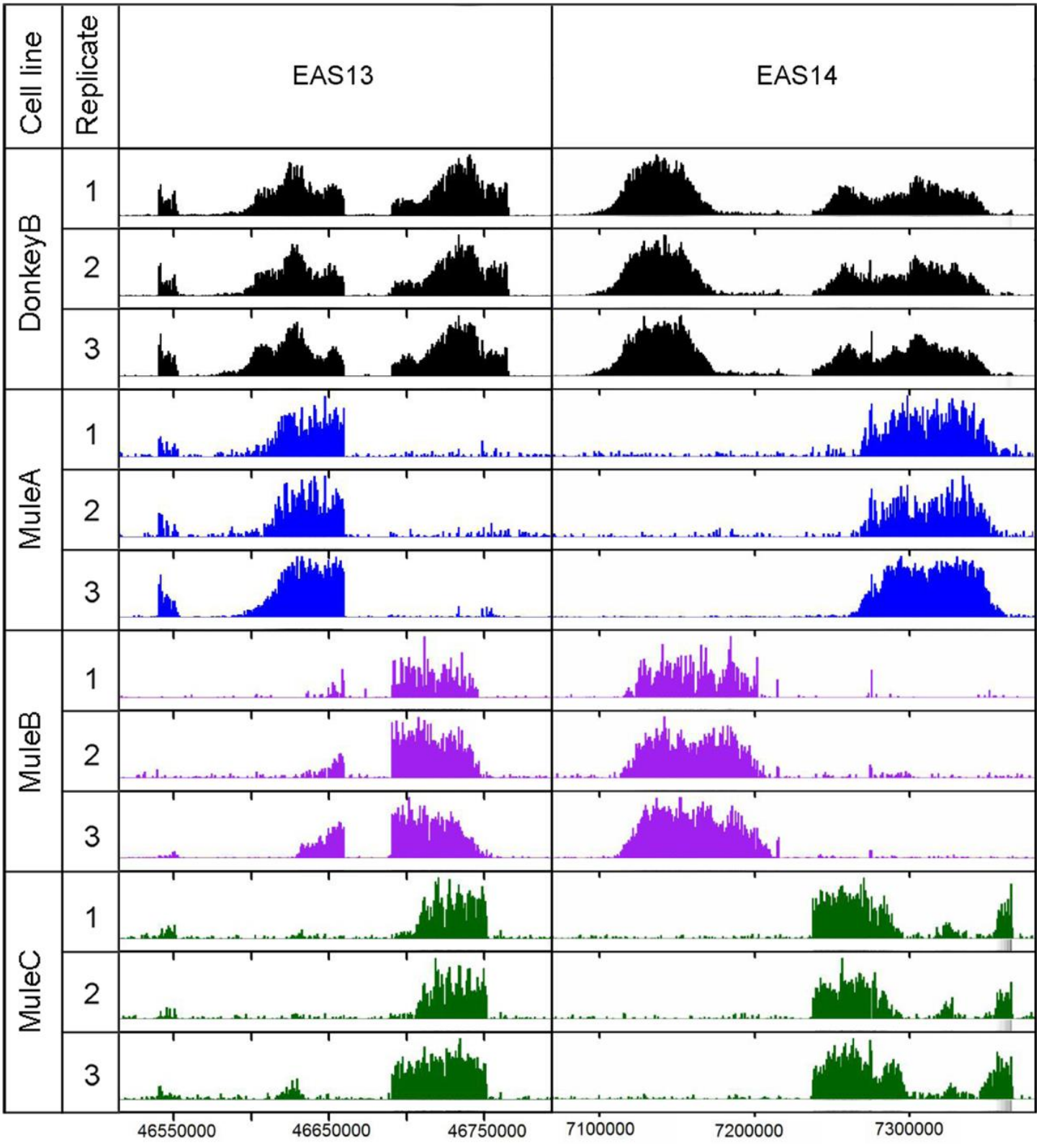
Family trees reporting the genetic relationships among the individuals used in this study are reported in Figure 4A. ChIP-seq analyses performed with the anti-CENP-A antibody on chromatin from the DonkeyB cell line and the cell lines from its offspring MuleA, MuleB and MuleC are shown. For each cell line the results of three experiments are reported. The peaks from donkey chromosomes not reported in Figure 4B are shown (EAS5, 10, 11, 12, 13, 14, 27 and 30). In addition, inheritance of the centromeric domains of horse chromosome 11, in the same three mules and in the hinny from the second family (Figure 4A), is shown.

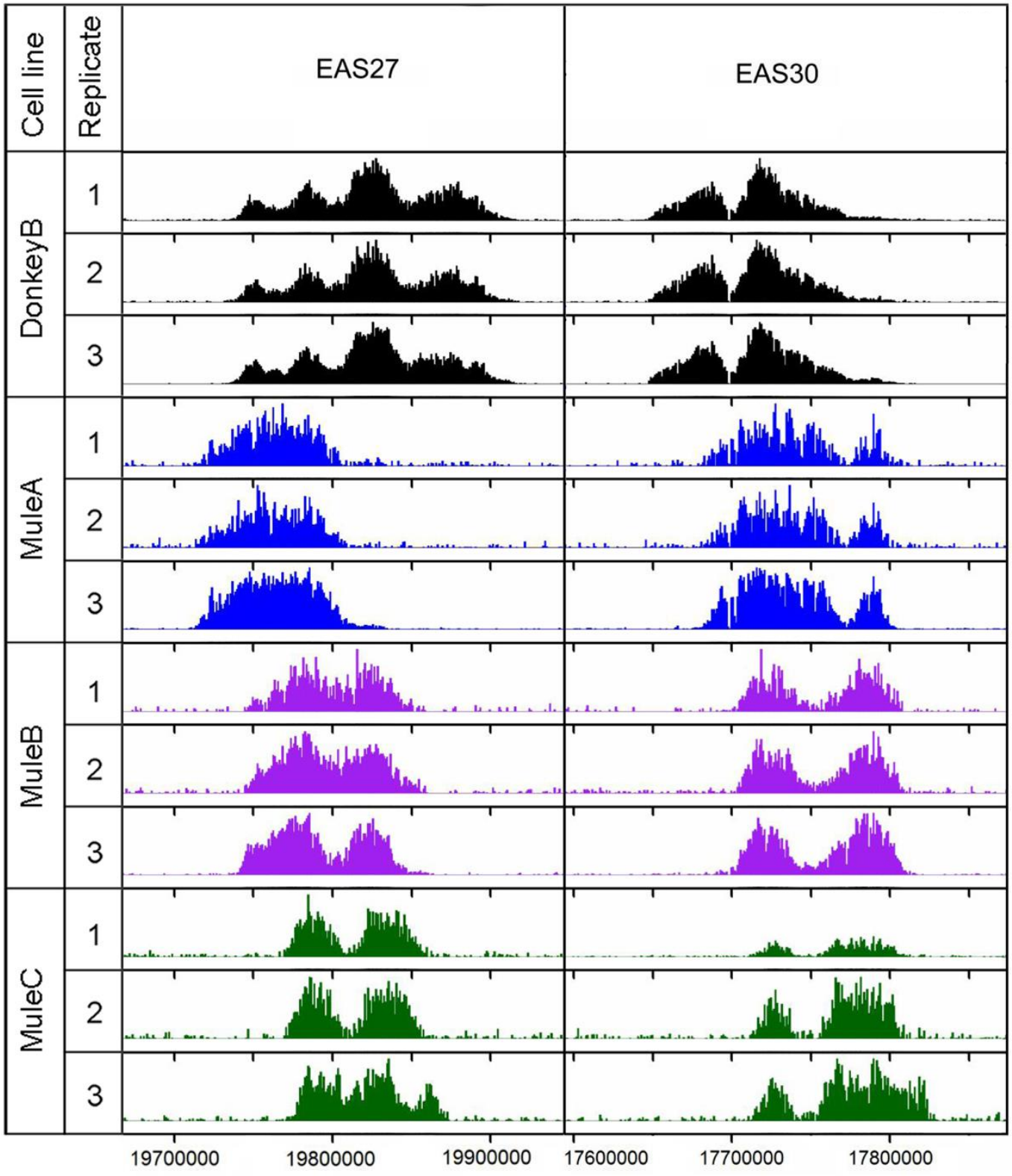
Figure S7


Cell line
Replicate
EAS5
DonkeyB
1
2
3
MuleA
1
2
3
MuleB
1
2
3
MuleC
1
2
3
4600000
4700000
4800000
4900000
5000000


Cell line
Replicate
EAS10
EAS11
DonkeyB
1
2
3
MuleA
1
2
3
MuleB
1
2
3
MuleC
1
2
3
8500000
8600000
8700000
16700000
16800000


Cell line
Replicate
EAS12
DonkeyB
1
2
3
MuleA
1
2
3
MuleB
1
2
3
MuleC
1
2
3
31600000
31700000
31800000
31900000
32000000


Cell line
Replicate
EAS13
EAS14
DonkeyB
MuleA
MuleB
MuleC
1
2
3
46550000
46650000
46750000
7100000
7200000
7300000


Cell line
Replicate
EAS27
EAS30
DonkeyB
MuleA
MuleB
MuleC
1
2
3
19700000
19800000
19900000
17600000
17700000
17800000

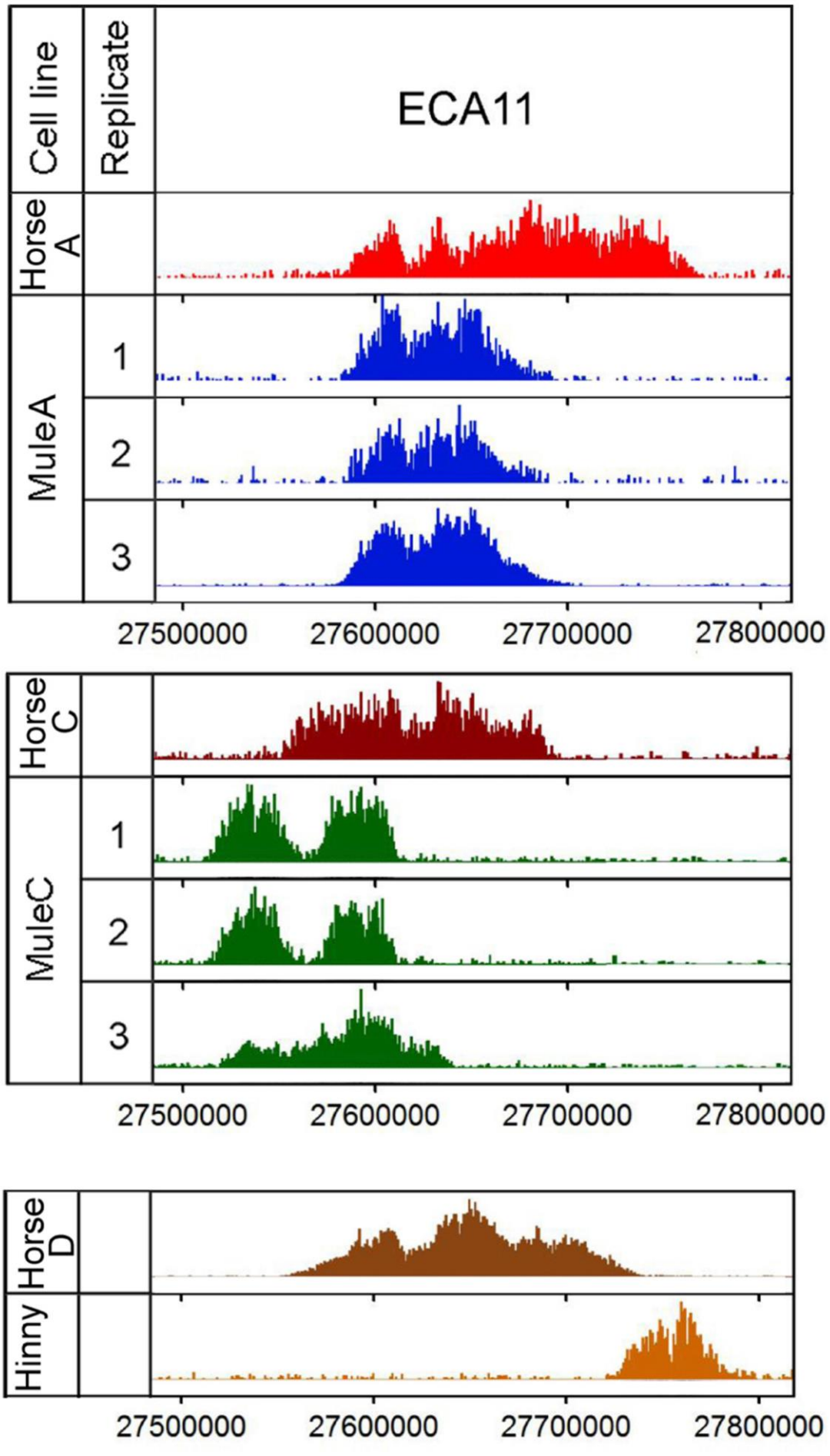

Cell line
Replicate
ECA11
Horse A
MuleA
1
2
3
27500000
27600000
27700000
27800000
Horse C
MuleC
1
2
3
27500000
27600000
27700000
27800000
Horse D
Hinny
27500000
27600000
27700000
27800000

Figure S8: Transmission of satellite-less centromeric domains in clonal cell lines

The results of ChIP-seq analysis from the immortalized cell line obtained from MuleA primary fibroblasts and six clonal derivative cell lines are shown. The centromeric domains of the chromosomes not shown in Figure 5 are reported here (EAS5, 10, 12, 13, 14, 27 and 30). As for Figure 4B, the EquCabAsiB chimeric genome was used as reference.

Figure S8

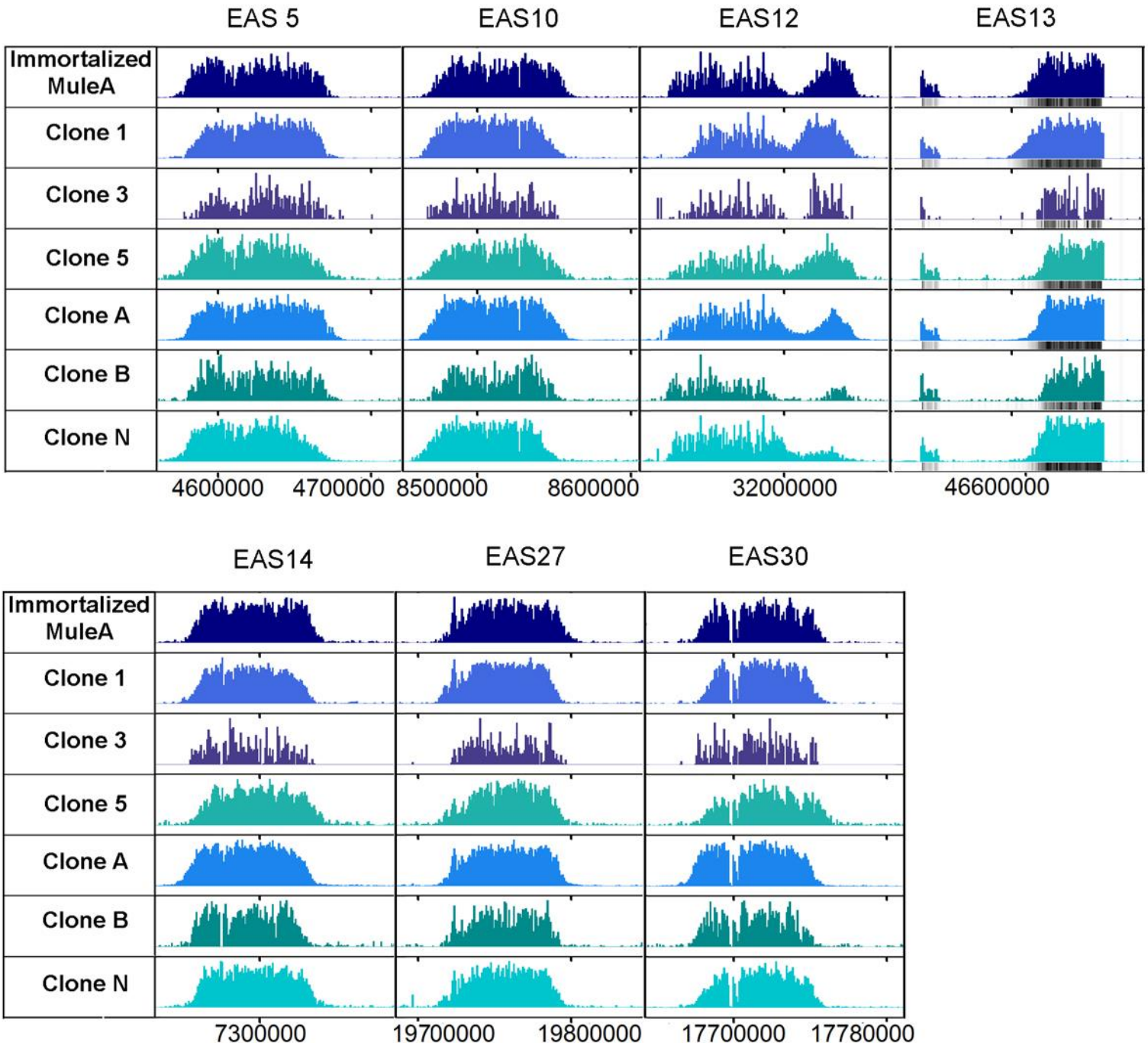

EAS 5
EAS10
EAS12
EAS13
Immortalized MuleA
Clone 1
Clone 3
Clone 5
Clone A
Clone B
Clone N
4600000
4700000
8500000
8600000
32000000
46600000
EAS14
EAS27
EAS30
Immortalized MuleA
Clone 1
Clone 3
Clone 5
Clone A
Clone B
Clone N
7300000
19700000
19800000
17700000
17780000

Table S1: Read counts and alignment statistics of ChIP-seq experiments

SAMPLE	Total reads	Mapped reads	Paired reads	Read length	Reference genome
HorseS CREST serum	77706974	62418704	55222324	100 bp	EquCab2.0
HorseS anti-CENPA	78207302	71308045	68006870	100 bp	EquCab2.0
HorseS INPUT	41155660	39319169	38983866	100 bp	EquCab2.0
DonkeyA CREST serum	77041628	67047335	64108564	100 bp	EquCab2.0
DonkeyA anti-CENPA	28937922	22643915	21186936	100 bp	EquCab2.0
DonkeyA INPUT	247035998	217757825	211754962	100 bp	EquCab2.0
DonkeyB ChIP rep1	44267364	41222372	38636682	100 bp	EquCabAsiB
DonkeyB input rep1	37434334	35059573	32856522	100 bp	EquCabAsiB
DonkeyB ChIP rep2	20012978	18390439	16186294	125 bp	EquCabAsiB
DonkeyB ChIP rep3	22546630	20885494	16334088	125 bp	EquCabAsiB
DonkeyB input rep3	25308774	23372420	18665452	125 bp	EquCabAsiB
HorseA ChIP	39468546	37862819	35629552	100 bp	EquCabAsiB
HorseA input	51543286	50403553	48347458	100 bp	EquCabAsiB
HorseC ChIP	42683528	40775785	37105694	100 bp	EquCabAsiB
HorseC input	45821170	44894301	42988808	100 bp	EquCabAsiB
MuleA ChIP rep1	22346700	20643041	18279998	100 bp	EquCabAsiB
MuleA input rep1	44567226	42273515	40024402	100 bp	EquCabAsiB
MuleA ChIP rep2	23579194	21661251	20729224	125 bp	EquCabAsiB
MuleA ChIP rep3	54609440	51511015	41026558	125 bp	EquCabAsiB
MuleA input rep3	22879084	21454167	17690014	125 bp	EquCabAsiB
MuleB ChIP rep1	62852362	39317523	3300190	100 bp	EquCabAsiB
MuleB input rep1	42439894	40244505	37340448	100 bp	EquCabAsiB
MuleB ChIP rep2	36288682	25037131	20075340	125 bp	EquCabAsiB
MuleB ChIP rep3	21825944	20650179	18946480	125 bp	EquCabAsiB
MuleB input rep3	24923270	23564444	19986172	125 bp	EquCabAsiB
MuleC ChIP rep1	48598812	44951839	41422772	100 bp	EquCabAsiB
MuleC input rep1	52076548	49169746	43301750	100 bp	EquCabAsiB
MuleC ChIP rep2	22904772	21210009	19859576	125 bp	EquCabAsiB
MuleC ChIP rep3	23177822	21638463	17782174	125 bp	EquCabAsiB
MuleC input rep3	19584370	18488908	15593418	125 bp	EquCabAsiB
MuleA Immortal ChIP	40779044	38415453	33635982	125 bp	EquCabAsiB
MuleA Immortal input	34772184	32766378	28821438	125 bp	EquCabAsiB
MuleA Immortal clone1 ChIP	44974168	42853652	40591650	100 bp	EquCabAsiB
MuleA Immortal clone 1 input	79649436	76121539	71318044	100 bp	EquCabAsiB
MuleA Immortal clone 3 ChIP	39754672	33605866	31891924	100 bp	EquCabAsiB
MuleA Immortal clone 3 input	112073498	107287681	101937526	100 bp	EquCabAsiB

MuleA Immortal clone 5 ChIP	38358616	36456292	30781156	125 bp	EquCabAsiB
MuleA Immortal clone 5 input	37197058	34317971	29862728	125 bp	EquCabAsiB
MuleA Immortal clone A ChIP	42946392	40245858	37205790	100 bp	EquCabAsiB
MuleA Immortal clone A input	67218108	64111271	60395772	100 bp	EquCabAsiB
MuleA Immortal clone B ChIP	31542160	29743439	26089810	125 bp	EquCabAsiB
MuleA Immortal clone B input	39628692	37294493	32330984	125 bp	EquCabAsiB
MuleA Immortal clone N ChIP	27428308	26158240	24477532	100 bp	EquCabAsiB
MuleA Immortal clone N input	68493230	64544804	61541167	100 bp	EquCabAsiB
HorseD ChIP	42913544	41804917	39517726	100 bp	EquCab2.0
HorseD Input	40185508	38776515	35386124	100 bp	EquCab2.0
Hinny ChIP	33216746	31522918	29762034	100 bp	EquCab2.0
Hinny Input	46155838	43873637	40619456	100 bp	EquCab2.0

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Table S2: BAC clones from the CH241 library spanning the horse genome regions enriched by ChIP-seq

Donkey chromosome	BAC code	NCBI EquCab2.0
EAS4	CH241-336L20	chr28:12,847,872-13,039,734 nt
	CH241-428I12	chr28:12,869,899-13,052,689 nt
EAS5	CH241-20K22	chr19:4,913,928-5,070,194 nt
	CH241-120M19	chr19:4,961,410-5,133,574 nt
EAS7	CH241-387D1	chr8:41,977,836-42,179,817 nt
	CH241-402C18	chr8:41,977,313-42,179,897 nt
EAS8	CH241-259C15	chr20:26,398,987-26,598,744 nt
	CH241-80D14	chr20:26,405,502-26,563,491 nt
EAS9	CH241-165K13	chr14:29,577,466-29,748,638 nt
	CH241-377E16	chr14:29,599,697-29,806,985 nt
EAS10	CH241-115N8	chr25:8,531,817-8,705,624 nt
	CH241-121M13	chr25:8,702,977-8,879,373 nt
EAS11	CH241-134J4	chr17:16,694,007-16,890,618 nt
EAS12	CH241-399M14	chr9:31,826,521-32,005,467 nt
	CH241-278F3	chr9:32,158,931-32,339,912 nt
EAS13	CH241-232I17	chr11:46,749,358-46,973,150 nt
	CH241-216D6	chr11:46,602,814-46,770,785 nt
EAS14	CH241-1G14	chr13:7,217,109-7,405,642 nt
	CH241-22C1	chr13:7,346,775-7,544,907 nt
EAS16	CH241-391H10	chr5:74,802,205-75,007,031 nt
EAS18	CH241-301P23	chr26:22,357,611-22,542,674 nt
EAS19	CH241-280L13	chr6:14,128,476-14,305,472 nt
EAS27	CH241-336I24	chr27:19,650,895-19,837,418 nt
EAS30	CH241-132M24	chr30:17,711,329-17,873,536 nt
EASX	CH241-238L8	chrX: 26,951,983-27,103,770 nt

Table S3: Information on the assembly of the two chimeric reference genomes EquCabAsiA and EquCabAsiB
* number of *de novo* sequenced nucleotides

Chromosome		EquCabAsiA				EquCabAsiB			
		Length of contig (bp)*	Region removed from EquCab2.0			Length of contig (bp)*	Region removed from EquCab2.0		
Donkey	Horse		start	end	length (bp)		start	end	length (bp)
EAS4	ECA28	213274	12857498	13067693	210195	460942	12904279	13466657	562378
EAS5	ECA19	288763	4870728	5193891	323163	484788	4550152	5163572	613420
EAS7	ECA8	264091	41899430	42195191	295761	297039	41817689	42137502	319813
EAS8	ECA20	216802	26350299	26600000	249701	-	-	-	-
EAS9	ECA14	247803	29550027	29850000	299973	64666	29549976	29860001	310025
EAS10	ECA25	326839	8536970	8900000	363030	276056	8466521	8749564	283043
EAS11	ECA17	197947	16699392	16923707	224315	119276	16757631	16877565	119934
EAS12	ECA9	337945	31929515	32332937	403422	454369	31584316	32145282	560966
EAS13	ECA11	229638	46659851	46917380	257529	193144	46556884	46917446	360562
EAS14	ECA13	357906	7178188	7596941	418753	343246	7086710	7470346	383636
EAS16	ECA5	156176	74800027	74994518	194491	127003	74879061	75027728	148667
EAS18	ECA26	219675	22328608	22543315	214707	244030	22276704	22535514	258810
EAS19	ECA6	167346	14124005	14305040	181035	152013	14177149	14323090	145941
EAS27	ECA27	301893	19665636	19975899	310263	201316	19712912	19918612	205700
EAS30	ECA30	156951	17703305	17872653	169348	188741	17636535	17838722	202187
EASX	ECAX	204309	26900001	27139288	239287	125707	26942648	27073635	130987

Table S4: Sequence of primer pairs used in the different PCR experiments	
Sequence 5'-3'	**Application**
TAGGAGGGAGATCTGGGAGCTTG CTTTCCTGTGTCTCACTTTCATTATAGCTG	Amplification of Southern blot probe for EAS9
GCTTTCACTTAGGTTTGCCAATG AGTTCAATTGTTGGCTGTTGGA	Amplification of Southern blot probe for EAS18
GAGCATTTGATTGCAAGAACC CAGCTGATGACTTGCCACCA	Amplification of Southern blot probe for EAS19
GAAAGGAGCAGCATTCTATGGTC GGAAGAGGAAATACTGAGAAACGC	Single-copy control region in the qPCR assays
TCTACGGGATATTCCACCAATG GAATTTGGAACTCTGGACTTTGG	qPCR assay on EAS9-ECA14
CTCCTTCACGACTATTTCCACAC CTTGGAGCTGATGTTTATCCGA	qPCR assay on EAS9-ECA14
TGGTTGGCATCAAATTCTATGGT GATAACATGAGCTGTTGCCCTT	qPCR assay on EAS18-ECA26
GTTAAATCAAGGTGGCCCTCAGT AGTTGTTTGGGCAGTGTTTATTG	qPCR assay on EAS18-ECA26
GTTTTCCCACCCTAAAAGCATCT GAGTTCCCAACAAAAGAAGTGC	qPCR assay on EAS19-ECA6
TGTTTCTATATACCGCAAAGGTCG CAGCTGATGACTTGCCACCA	qPCR assay on EAS19-ECA6
ATGACTGAAGATCCATCCTCTGAA GAACATTTGAGTATGGAGCCTGA	qPCR assay on EAS8-ECA20
TCTCCAACAGACCTCAGCTAGAAAC AGGCGAGGATCCAAAACTGA	qPCR assay on EAS8-ECA20
CCTGTGTCTCTTGCAAGATCACTT GGTTGGAGACATAAGCATAACAATTC	qPCR assay on EAS8-ECA20
TATGAACTTGGTCCAGGCTATGA CCTCCTACCTTTGCACCTTTG	qPCR assay on EAS8-ECA20
CGGACATGGCACCACTCA CATGAGCTAACATTTGCTGCCT	qPCR assay on EAS16-ECA5
TCTGACAGCCATTTCATTTGGTG CAATAACTCAGAAGGGGAAGTTGG	qPCR assay on EAS16-ECA5
GTCTCTTATCTCCATGAGCCTGATGA ACTCTCTTTCAGAACAGGGAAGC	qPCR assay on EAS16-ECA5

Table S5: SNV analysis

				INPUT					CHIP				
Chr n° (EAS)	Chr n° (ECA)	Position	SNV	Tot. Reads	A (%)	C (%)	G (%)	T (%)	Tot. Reads	A (%)	C (%)	G (%)	T (%)
4	28	12,882,531	CT	13	0 (0)	6 (46,2)	0 (0)	7 (53,8)	7	0 (0)	0 (0)	0 (0)	7 (100)
4	28	12,883,276	AG	5	2 (40)	0 (0)	3 (60)	0 (0)	36	36 (100)	0 (0)	0 (0)	0 (0)
4	28	12,886,338	GT	9	0 (0)	0 (0)	4 (44,4)	5 (55,6)	4	0 (0)	0 (0)	3 (75)	1 (25)
4	28	12,896,097	AG	11	6 (54,5)	0 (0)	5 (45,5)	0 (0)	20	0 (0)	0 (0)	20 (100)	0 (0)
4	28	12,897,942	GT	7	0 (0)	0 (0)	4 (57,1)	3 (42,9)	11	0 (0)	0 (0)	11 (100)	0 (0)
4	28	12,899,763	AG	7	3 (42,9)	0 (0)	4 (57,1)	0 (0)	21	0 (0)	0 (0)	21 (100)	0 (0)
4	28	12,903,110	AT	10	4 (40)	0 (0)	0 (0)	6 (60)	10	0 (0)	0 (0)	0 (0)	10 (100)
4	28	12,917,192	CT	7	0 (0)	3 (42,9)	0 (0)	4 (57,1)	45	0 (0)	0 (0)	0 (0)	45 (100)
4	28	12,922,452	GT	6	0 (0)	0 (0)	3 (50)	3 (50)	50	0 (0)	0 (0)	50 (100)	0 (0)
4	28	12,924,566	GT	16	0 (0)	0 (0)	8 (50)	8 (50)	49	0 (0)	0 (0)	48 (98)	1 (2)
4	28	12,925,570	AG	11	5 (45,5)	0 (0)	6 (54,5)	0 (0)	29	0 (0)	0 (0)	29 (100)	0 (0)
4	28	12,936,801	AC	6	3 (50)	3 (50)	0 (0)	0 (0)	20	0 (0)	20 (100)	0 (0)	0 (0)
4	28	12,937,876	GT	5	0 (0)	0 (0)	3 (60)	2 (40)	12	0 (0)	0 (0)	12 (100)	0 (0)
4	28	12,945,682	CG	6	0 (0)	3 (50)	3 (50)	0 (0)	109	1 (0,9)	17 (15,6)	91 (83,5)	0 (0)
4	28	12,967,250	GT	14	0 (0)	0 (0)	8 (57,1)	6 (42,9)	61	0 (0)	0 (0)	29 (47,5)	32 (52,5)
4	28	12,970,871	CG	7	0 (0)	4 (57,1)	3 (42,9)	0 (0)	19	0 (0)	12 (63,2)	7 (36,8)	0 (0)
4	28	12,972,614	AG	10	4 (40)	0 (0)	6 (60)	0 (0)	16	10 (62,5)	0 (0)	6 (37,5)	0 (0)
4	28	12,978,797	CT	12	0 (0)	6 (50)	0 (0)	6 (50)	31	0 (0)	8 (25,8)	0 (0)	23 (74,2)
4	28	12,979,317	CT	12	0 (0)	7 (58,3)	0 (0)	5 (41,7)	58	1 (1,7)	45 (77,6)	0 (0)	12 (20,7)
4	28	12,988,866	CT	10	0 (0)	6 (60)	0 (0)	4 (40)	25	0 (0)	0 (0)	0 (0)	25 (100)
4	28	12,991,162	CG	15	0 (0)	9 (60)	6 (40)	0 (0)	46	0 (0)	5 (10,9)	41 (89,1)	0 (0)
4	28	12,992,643	CT	12	0 (0)	5 (41,7)	0 (0)	7 (58,3)	19	0 (0)	18 (94,7)	0 (0)	1 (5,3)
4	28	12,993,807	GT	10	0 (0)	0 (0)	6 (60)	4 (40)	11	0 (0)	0 (0)	11 (100)	0 (0)
4	28	13,005,492	GT	9	0 (0)	0 (0)	5 (55,6)	4 (44,4)	22	0 (0)	0 (0)	0 (0)	22 (100)
4	28	13,007,228	GT	10	0 (0)	0 (0)	5 (50)	5 (50)	31	0 (0)	0 (0)	2 (6,5)	29 (93,5)
4	28	13,014,024	GT	6	0 (0)	0 (0)	3 (50)	3 (50)	29	0 (0)	0 (0)	29 (100)	0 (0)
4	28	13,017,861	AC	7	3 (42,9)	4 (57,1)	0 (0)	0 (0)	51	47 (92,2)	4 (7,8)	0 (0)	0 (0)
4	28	13,021,185	AG	7	4 (57,1)	0 (0)	3 (42,9)	0 (0)	4	0 (0)	0 (0)	4 (100)	0 (0)
4	28	13,025,313	AC	12	5 (41,7)	7 (58,3)	0 (0)	0 (0)	20	1 (5)	19 (95)	0 (0)	0 (0)
4	28	13,040,917	AG	10	5 (50)	0 (0)	5 (50)	0 (0)	32	0 (0)	0 (0)	32 (100)	0 (0)
4	28	13,045,308	CT	9	0 (0)	4 (44,4)	0 (0)	5 (55,6)	6	0 (0)	6 (100)	0 (0)	0 (0)
4	28	13,051,620	AC	10	6 (60)	4 (40)	0 (0)	0 (0)	13	13 (100)	0 (0)	0 (0)	0 (0)

				INPUT					CHIP				
Chr n° (EAS)	Chr n° (ECA)	Position	SNV	Tot. Reads	A (%)	C (%)	G (%)	T (%)	Tot. Reads	A (%)	C (%)	G (%)	T (%)
5	19	4,923,058	GT	21	0 (0)	0 (0)	10 (47,6)	11 (52,4)	10	0 (0)	0 (0)	0 (0)	10 (100)
5	19	4,923,956	AC	14	6 (42,9)	8 (57,1)	0 (0)	0 (0)	19	0 (0)	19 (100)	0 (0)	0 (0)
5	19	4,924,636	GT	15	0 (0)	0 (0)	9 (60)	6 (40)	24	0 (0)	0 (0)	23 (95,8)	1 (4,2)
5	19	4,925,418	CT	29	0 (0)	13 (44,8)	0 (0)	16 (55,2)	10	0 (0)	10 (100)	0 (0)	0 (0)
5	19	4,925,528	CT	29	1 (3,5)	15 (51,7)	0 (0)	13 (44,8)	6	0 (0)	6 (100)	0 (0)	0 (0)
5	19	4,925,896	AG	22	11 (50)	0 (0)	11 (50)	0 (0)	18	1 (5,6)	0 (0)	17 (94,4)	0 (0)
5	19	4,926,124	AG	26	11 (42,3)	0 (0)	15 (57,7)	0 (0)	12	12 (100)	0 (0)	0 (0)	0 (0)
5	19	4,927,785	AC	23	12 (52,2)	11 (47,8)	0 (0)	0 (0)	19	0 (0)	19 (100)	0 (0)	0 (0)
5	19	4,929,414	GT	24	0 (0)	0 (0)	12 (50)	12 (50)	15	0 (0)	0 (0)	1 (6,7)	14 (93,3)
5	19	4,930,542	AG	12	6 (50)	0 (0)	6 (50)	0 (0)	15	0 (0)	0 (0)	15 (100)	0 (0)
5	19	4,934,161	GT	12	0 (0)	0 (0)	5 (41,7)	7 (58,3)	52	0 (0)	0 (0)	2 (3,8)	50 (96,2)
5	19	4,936,622	CT	4	0 (0)	2 (50)	0 (0)	2 (50)	66	0 (0)	66 (100)	0 (0)	0 (0)
5	19	4,941,972	AG	18	8 (44,4)	0 (0)	10 (55,6)	0 (0)	13	0 (0)	0 (0)	13 (100)	0 (0)
5	19	4,947,350	AC	6	3 (50)	3 (50)	0 (0)	0 (0)	42	1 (2,4)	41 (97,6)	0 (0)	0 (0)
5	19	4,948,610	CT	5	0 (0)	3 (60)	0 (0)	2 (40)	8	0 (0)	0 (0)	0 (0)	8 (100)
5	19	4,949,637	AG	17	9 (52,9)	0 (0)	8 (47,1)	0 (0)	19	0 (0)	0 (0)	19 (100)	0 (0)
5	19	4,950,605	CT	15	1 (6,6)	7 (46,7)	0 (0)	7 (46,7)	33	0 (0)	0 (0)	0 (0)	33 (100)
5	19	4,957,961	CT	8	0 (0)	4 (50)	0 (0)	4 (50)	20	0 (0)	1 (5)	0 (0)	19 (95)
5	19	4,965,140	AT	10	6 (60)	0 (0)	0 (0)	4 (40)	67	67 (100)	0 (0)	0 (0)	0 (0)
5	19	4,966,982	CT	13	0 (0)	7 (53,8)	0 (0)	6 (46,2)	12	0 (0)	12 (100)	0 (0)	0 (0)
5	19	4,968,443	AG	18	10 (55,6)	0 (0)	8 (44,4)	0 (0)	17	16 (94,1)	0 (0)	1 (5,9)	0 (0)
5	19	4,992,089	CT	8	0 (0)	4 (50)	0 (0)	4 (50)	5	0 (0)	5 (100)	0 (0)	0 (0)
5	19	5,002,476	CG	5	0 (0)	2 (40)	3 (60)	0 (0)	36	0 (0)	35 (97,2)	0 (0)	1 (2,8)
5	19	5,011,974	AG	7	4 (57,1)	0 (0)	3 (42,9)	0 (0)	12	11 (91,7)	0 (0)	1 (8,3)	0 (0)
5	19	5,018,143	CT	9	0 (0)	5 (55,6)	0 (0)	4 (44,4)	9	0 (0)	7 (77,8)	0 (0)	2 (22,2)
5	19	5,034,935	AG	10	5 (50)	0 (0)	5 (50)	0 (0)	5	0 (0)	0 (0)	5 (100)	0 (0)
5	19	5,038,363	CT	13	0 (0)	7 (53,8)	0 (0)	6 (46,2)	10	0 (0)	1 (10)	0 (0)	9 (90)
5	19	5,040,712	CT	16	0 (0)	7 (43,8)	0 (0)	9 (56,2)	14	0 (0)	0 (0)	0 (0)	14 (100)
5	19	5,040,955	GT	10	0 (0)	0 (0)	6 (60)	4 (40)	8	0 (0)	0 (0)	0 (0)	8 (100)

5	19	5,040,956	GT	9	0 (0)	0 (0)	4 (44,4)	5 (55,6)	8	0 (0)	0 (0)	8 (100)	0 (0)
5	19	5,045,359	AT	13	6 (46,2)	0 (0)	0 (0)	7 (53,8)	4	0 (0)	0 (0)	0 (0)	4 (100)
5	19	5,053,259	AG	12	6 (50)	0 (0)	6 (50)	0 (0)	71	0 (0)	0 (0)	71 (100)	0 (0)
5	19	5,053,672	CG	12	0 (0)	7 (58,3)	5 (41,7)	0 (0)	40	0 (0)	40 (100)	0 (0)	0 (0)
5	19	5,056,031	CT	18	0 (0)	10 (55,6)	0 (0)	8 (44,4)	42	0 (0)	42 (100)	0 (0)	0 (0)
5	19	5,060,155	AC	9	5 (55,6)	4 (44,4)	0 (0)	0 (0)	49	0 (0)	49 (100)	0 (0)	0 (0)
5	19	5,060,263	CG	9	1 (11,2)	4 (44,4)	4 (44,4)	0 (0)	42	0 (0)	42 (100)	0 (0)	0 (0)
5	19	5,060,547	AT	10	6 (60)	0 (0)	0 (0)	4 (40)	6	6 (100)	0 (0)	0 (0)	0 (0)
5	19	5,061,881	AT	9	5 (55,6)	0 (0)	0 (0)	4 (44,4)	29	1 (3,4)	0 (0)	0 (0)	28 (96,6)
5	19	5,063,137	AG	10	4 (40)	0 (0)	6 (60)	0 (0)	38	0 (0)	0 (0)	38 (100)	0 (0)
5	19	5,071,107	AG	11	5 (45,5)	0 (0)	6 (54,5)	0 (0)	13	13 (100)	0 (0)	0 (0)	0 (0)
5	19	5,074,866	AG	15	7 (46,7)	0 (0)	8 (53,3)	0 (0)	25	25 (100)	0 (0)	0 (0)	0 (0)
5	19	5,105,840	AC	15	8 (53,3)	7 (46,7)	0 (0)	0 (0)	40	40 (100)	0 (0)	0 (0)	0 (0)
5	19	5,107,849	CT	14	0 (0)	7 (50)	0 (0)	7 (50)	41	0 (0)	0 (0)	0 (0)	41 (100)
5	19	5,111,743	AC	5	2 (40)	3 (60)	0 (0)	0 (0)	42	41 (97,6)	1 (2,4)	0 (0)	0 (0)
5	19	5,111,984	AG	10	6 (60)	0 (0)	4 (40)	0 (0)	42	42 (100)	0 (0)	0 (0)	0 (0)
5	19	5,115,660	CT	15	0 (0)	9 (60)	0 (0)	6 (40)	21	0 (0)	21 (100)	0 (0)	0 (0)
5	19	5,116,698	GT	8	0 (0)	0 (0)	4 (50)	4 (50)	15	0 (0)	0 (0)	1 (6,7)	14 (93,3)
5	19	5,118,238	GT	14	0 (0)	0 (0)	7 (50)	7 (50)	29	0 (0)	0 (0)	29 (100)	0 (0)
5	19	5,120,135	GT	11	0 (0)	0 (0)	6 (54,5)	5 (45,5)	33	0 (0)	0 (0)	0 (0)	33 (100)
5	19	5,125,168	CT	12	0 (0)	6 (50)	0 (0)	6 (50)	5	0 (0)	0 (0)	0 (0)	5 (100)
5	19	5,127,833	CG	9	0 (0)	4 (44,4)	5 (55,6)	0 (0)	21	0 (0)	21 (100)	0 (0)	0 (0)
5	19	5,134,035	CT	13	0 (0)	7 (53,8)	0 (0)	6 (46,2)	10	0 (0)	10 (100)	0 (0)	0 (0)
5	19	5,135,340	AC	8	4 (50)	4 (50)	0 (0)	0 (0)	8	8 (100)	0 (0)	0 (0)	0 (0)

				INPUT					CHIP				
Chr n° (EAS)	Chr n° (ECA)	Position	SNV	Tot. Reads	A (%)	C (%)	G (%)	T (%)	Tot. Reads	A (%)	C (%)	G (%)	T (%)
7	8	42,023,117	GT	12	0 (0)	0 (0)	6 (50)	6 (50)	25	0 (0)	0 (0)	10 (40)	15 (60)
7	8	42,028,016	CT	21	0 (0)	9 (42,9)	0 (0)	12 (57,1)	89	0 (0)	39 (43,8)	0 (0)	50 (56,2)
7	8	42,046,795	GT	16	0 (0)	0 (0)	9 (56,3)	7 (43,7)	43	0 (0)	0 (0)	43 (100)	0 (0)
7	8	42,071,123	CT	17	0 (0)	9 (52,9)	0 (0)	8 (47,1)	18	0 (0)	16 (88,9)	0 (0)	2 (11.1)
7	8	42,076,829	GT	15	0 (0)	1 (6,7)	6 (40)	8 (53,3)	36	0 (0)	0 (0)	9 (25)	27 (75)

				INPUT					CHIP				
Chr n° (EAS)	Chr n° (ECA)	Position	SNV	Tot. Reads	A (%)	C (%)	G (%)	T (%)	Tot. Reads	A (%)	C (%)	G (%)	T (%)
10	25	8,595,609	CG	10	0 (0)	6 (60)	4 (40)	0 (0)	19	0 (0)	19 (100)	0 (0)	0 (0)
10	25	8,630,560	AT	17	9 (52,9)	0 (0)	0 (0)	8 (47,1)	19	19 (100)	0 (0)	0 (0)	0 (0)
10	25	8,635,514	CG	15	0 (0)	6 (40)	9 (60)	0 (0)	56	0 (0)	0 (0)	56 (100)	0 (0)
10	25	8,640,609	AC	7	3 (42,9)	4 (57,1)	0 (0)	0 (0)	50	0 (0)	50 (100)	0 (0)	0 (0)
10	25	8,642,694	CT	6	0 (0)	3 (50)	0 (0)	3 (50)	21	0 (0)	0 (0)	0 (0)	21 (100)
10	25	8,661,413	GT	7	0 (0)	0 (0)	3 (42,9)	4 (57,1)	36	0 (0)	0 (0)	36 (100)	0 (0)
10	25	8,668,968	AT	11	6 (54,5)	0 (0)	0 (0)	5 (45,5)	18	18 (100)	0 (0)	0 (0)	0 (0)
10	25	8,673,317	CT	6	0 (0)	3 (50)	0 (0)	3 (50)	5	0 (0)	0 (0)	0 (0)	5 (100)
10	25	8,678,064	AC	7	4 (57,1)	3 (42,9)	0 (0)	0 (0)	5	0 (0)	5 (100)	0 (0)	0 (0)
10	25	8,680,967	AC	9	5 (55,6)	4 (44,4)	0 (0)	0 (0)	39	39 (100)	0 (0)	0 (0)	0 (0)
10	25	8,681,460	AC	12	5 (41,7)	7 (58,3)	0 (0)	0 (0)	4	4 (100)	0 (0)	0 (0)	0 (0)
10	25	8,685,416	CT	9	0 (0)	4 (44,4)	0 (0)	5 (55,6)	13	0 (0)	13 (100)	0 (0)	0 (0)
10	25	8,687,130	CT	13	0 (0)	7 (53,8)	0 (0)	6 (46,2)	9	0 (0)	9 (100)	0 (0)	0 (0)
10	25	8,689,947	CG	8	0 (0)	4 (50)	4 (50)	0 (0)	7	0 (0)	0 (0)	7 (100)	0 (0)
10	25	8,689,716	AG	12	6 (50)	0 (0)	6 (50)	0 (0)	4	0 (0)	0 (0)	4 (100)	0 (0)
10	25	8,714,440	GT	10	0 (0)	0 (0)	4 (40)	6 (60)	5	0 (0)	0 (0)	5 (100)	0 (0)
10	25	8,714,549	AC	12	6 (50)	6 (50)	0 (0)	0 (0)	8	7 (87,5)	1 (12,5)	0 (0)	0 (0)
10	25	8,720,836	CG	8	0 (0)	4 (50)	4 (50)	0 (0)	30	0 (0)	0 (0)	30 (100)	0 (0)
10	25	8,722,477	CT	10	0 (0)	4 (40)	0 (0)	6 (60)	22	0 (0)	0 (0)	0 (0)	22 (100)
10	25	8,727,337	CT	9	0 (0)	4 (44,4)	0 (0)	5 (55,6)	45	0 (0)	45 (100)	0 (0)	0 (0)
10	25	8,747,841	GT	10	0 (0)	0 (0)	6 (60)	4 (40)	30	0 (0)	0 (0)	30 (100)	0 (0)
10	25	8,757,182	CT	9	0 (0)	4 (44,4)	0 (0)	5 (55,6)	34	0 (0)	34 (100)	0 (0)	0 (0)
10	25	8,761,294	CT	14	0 (0)	8 (57,1)	0 (0)	6 (42,9)	27	0 (0)	25 (92,6)	0 (0)	2 (7,4)
10	25	8,770,996	CT	7	0 (0)	3 (42,9)	0 (0)	4 (57,1)	45	0 (0)	0 (0)	0 (0)	45 (100)
10	25	8,806,469	CT	14	0 (0)	6 (42,9)	0 (0)	8 (57,1)	8	0 (0)	0 (0)	0 (0)	8 (100)

				INPUT					CHIP				
Chr n° (EAS)	Chr n° (ECA)	Position	SNV	Tot. Reads	A (%)	C (%)	G (%)	T (%)	Tot. Reads	A (%)	C (%)	G (%)	T (%)
12	9	32,020,722	CT	16	0 (0)	8 (50)	0 (0)	8 (50)	12	0 (0)	0 (0)	0 (0)	12 (100)
12	9	32,024,869	CT	12	0 (0)	5 (41,7)	0 (0)	7 (58,3)	9	0 (0)	0 (0)	0 (0)	9 (100)
12	9	32,034,237	AG	9	4 (44,4)	0 (0)	5 (55,6)	0 (0)	9	0 (0)	0 (0)	9 (100)	0 (0)
12	9	32,139,073	AG	5	3 (60)	0 (0)	2 (40)	0 (0)	9	0 (0)	0 (0)	9 (100)	0 (0)
12	9	32,139,215	AG	6	3 (50)	0 (0)	3 (50)	0 (0)	6	0 (0)	0 (0)	6 (100)	0 (0)
12	9	32,149,162	CT	8	0 (0)	4 (50)	0 (0)	4 (50)	13	0 (0)	0 (0)	0 (0)	13 (100)

12	9	32,153,503	AG	5	3 (60)	0 (0)	2 (40)	0 (0)	9	0 (0)	0 (0)	9 (100)	0 (0)
12	9	32,153,632	GT	13	0 (0)	0 (0)	7 (53,8)	6 (46,2)	4	0 (0)	0 (0)	0 (0)	4 (100)
12	9	32,161,749	AT	4	2 (50)	0 (0)	0 (0)	2 (50)	25	25 (100)	0 (0)	0 (0)	0 (0)
12	9	32,174,852	CT	10	0 (0)	5 (50)	0 (0)	5 (50)	20	0 (0)	0 (0)	0 (0)	20 (100)
12	9	32,183,634	CT	9	0 (0)	4 (44,4)	0 (0)	5 (55,6)	76	0 (0)	76 (100)	0 (0)	0 (0)
12	9	32,185,046	AG	9	5 (55,6)	0 (0)	4 (44,4)	0 (0)	67	64 (95,5)	0 (0)	3 (4,5)	0 (0)
12	9	32,213,361	CG	13	0 (0)	8 (53,8)	6 (46,2)	0 (0)	95	1 (1)	1 (1,1)	92 (97,9)	0 (0)
12	9	32,215,437	CT	15	0 (0)	9 (60)	0 (0)	6 (40)	5	0 (0)	0 (0)	0 (0)	5 (100)
12	9	32,217,259	CT	7	0 (0)	4 (57,1)	0 (0)	3 (42,9)	35	0 (0)	35 (100)	0 (0)	0 (0)

				INPUT					CHIP				
Chr n° (EAS)	Chr n° (ECA)	Position	SNV	Tot. Reads	A (%)	C (%)	G (%)	T (%)	Tot. Reads	A (%)	C (%)	G (%)	T (%)
14	13	7,223,199	CT	23	0 (0)	13 (56,5)	0 (0)	10 (43,5)	24	0 (0)	19 (79,2)	0 (0)	5 (20,8)
14	13	7,225,919	CG	10	0 (0)	6 (60)	4 (40)	0 (0)	22	0 (0)	19 (86,4)	3 (13,6)	0 (0)
14	13	7,226,840	CT	18	0 (0)	8 (44,4)	0 (0)	10 (55,6)	30	0 (0)	29 (96,7)	0 (0)	1(3,3)
14	13	7,251,893	CG	21	0 (0)	9 (42,9)	12 (57,1)	0 (0)	103	0 (0)	100 (97,1)	3 (2,9)	0 (0)
14	13	7,263,337	CG	20	0 (0)	12 (60)	8 (40)	0 (0)	36	0 (0)	36 (100)	0 (0)	0 (0)
14	13	7,278,757	CT	23	0 (0)	12 (52,2)	0 (0)	11 (47,8)	96	0 (0)	1 (1,1)	0 (0)	95 (98,9)
14	13	7,296,721	CT	23	0 (0)	11 (47,8)	0 (0)	12 (52,2)	32	0 (0)	0 (0)	0 (0)	32 (100)
14	13	7,297,230	CG	21	0 (0)	10 (47,6)	11 (52,4)	0 (0)	47	0 (0)	46 (97,9)	1 (2,1)	0 (0)
14	13	7,361,890	CG	17	0 (0)	8 (47,1)	8 (47,1)	1 (5,8)	52	0 (0)	12 (23,1)	40 (76,9)	0 (0)
14	13	7,422,645	GT	10	0 (0)	0 (0)	6 (60)	4 (40)	76	0 (0)	0 (0)	0 (0)	76 (100)

				INPUT					CHIP				
Chr n° (EAS)	Chr n° (ECA)	Position	SNV	Tot. Reads	A (%)	C (%)	G (%)	T (%)	Tot. Reads	A (%)	C (%)	G (%)	T (%)
27	27	19,716,662	AG	12	7 (58,3)	0 (0)	5 (41,7)	0 (0)	11	1 (9,1)	0 (0)	10 (90,9)	0 (0)
27	27	19,724,990	AT	8	4 (50)	0 (0)	0 (0)	4 (50)	11	11 (100)	0 (0)	0 (0)	0 (0)
27	27	19,730,695	CG	12	0 (0)	7 (58,3)	5 (41,7)	0 (0)	13	0 (0)	0 (0)	13 (100)	0 (0)
27	27	19,815,032	CT	18	0 (0)	9 (50)	0 (0)	9 (50)	34	0 (0)	20 (58,8)	0 (0)	14 (41,2)
27	27	19,815,418	GT	13	0 (0)	0 (0)	7 (53,8)	6 (46,2)	23	0 (0)	0 (0)	9 (39,1)	14 (60,9)
27	27	19,818,000	CT	11	0 (0)	6 (54,5)	0 (0)	5 (45,5)	59	0 (0)	28 (47,5)	0 (0)	31 (52,5)
27	27	19,824,098	AC	11	6 (54,5)	5 (45,5)	0 (0)	0 (0)	18	9 (50)	9 (50)	0 (0)	0 (0)
27	27	19,824,229	CT	12	0 (0)	5 (41,7)	0 (0)	7 (58,3)	28	0 (0)	19 (67,9)	0 (0)	9 (32,1)
27	27	19,825,942	AT	8	4 (50)	0 (0)	0 (0)	4 (50)	60	40 (66,7)	0 (0)	0 (0)	20 (33,3)
27	27	19,826,921	AG	10	4 (40)	0 (0)	6 (60)	0 (0)	28	17 (60,7)	0 (0)	11 (39,3)	0 (0)
27	27	19,827,307	CT	12	0 (0)	6 (50)	0 (0)	6 (50)	27	0 (0)	14 (51,9)	0 (0)	13 (48,1)
27	27	19,830,065	CT	12	0 (0)	7 (58,3)	0 (0)	5 (41,7)	38	0 (0)	30 (78,9)	0 (0)	8 (21,1)
27	27	19,841,023	AG	14	8 (57,1)	0 (0)	6 (42,9)	0 (0)	27	22 (81,5)	0 (0)	5 (18,5)	0 (0)
27	27	19,849,900	CT	8	0 (0)	4 (50)	0 (0)	4 (50)	39	0 (0)	39 (100)	0 (0)	0 (0)
27	27	19,849,923	CT	11	0 (0)	6 (54,5)	0 (0)	5 (45,5)	47	0 (0)	0 (0)	0 (0)	47 (100)
27	27	19,850,899	GT	8	0 (0)	0 (0)	4 (50)	4 (50)	12	0 (0)	0 (0)	0 (0)	12 (100)
27	27	19,857,015	CG	10	0 (0)	6 (60)	4 (40)	0 (0)	43	0 (0)	43 (100)	0 (0)	0 (0)
27	27	19,866,550	AG	13	7 (53,8)	0 (0)	6 (46,2)	0 (0)	22	0 (0)	0 (0)	22 (100)	0 (0)
27	27	19,868,894	CT	15	0 (0)	8 (53,3)	0 (0)	7 (46,7)	30	0 (0)	30 (100)	0 (0)	0 (0)
27	27	19,876,728	GT	10	0 (0)	0 (0)	6 (60)	4 (40)	47	0 (0)	0 (0)	47 (100)	0 (0)
27	27	19,883,262	GT	13	0 (0)	0 (0)	7 (53,8)	6 (46,2)	45	0 (0)	0 (0)	0 (0)	45 (100)
27	27	19,884,542	AG	10	6 (60)	0 (0)	4 (40)	0 (0)	31	31 (100)	0 (0)	0 (0)	0 (0)
27	27	19,887,129	AC	8	4 (50)	4 (50)	0 (0)	0 (0)	45	45 (100)	0 (0)	0 (0)	0 (0)
27	27	19,890,742	AC	5	3 (60)	2 (40)	0 (0)	0 (0)	20	0 (0)	20 (100)	0 (0)	0 (0)
27	27	19,907,316	GT	7	0 (0)	0 (0)	3 (42,9)	4 (57,1)	13	0 (0)	0 (0)	0 (0)	13 (100)

				INPUT					CHIP				
Chr n° (EAS)	Chr n° (ECA)	Position	SNV	Tot. Reads	A (%)	C (%)	G (%)	T (%)	Tot. Reads	A (%)	C (%)	G (%)	T (%)
30	30	17,706,286	CT	5	0 (0)	2 (40)	0 (0)	3 (60)	8	0 (0)	7 (87,5)	0 (0)	1 (12,5)
30	30	17,713,101	AG	8	4 (50)	0 (0)	4 (50)	0 (0)	7	6 (85,7)	0 (0)	1 (14,3)	0 (0)
30	30	17,716,244	AG	8	4 (50)	0 (0)	4 (50)	0 (0)	15	7 (46,7)	0 (0)	8 (53,3)	0 (0)
30	30	17,718,586	CG	14	0 (0)	7 (50)	7 (50)	0 (0)	13	0 (0)	6 (46,2)	7 (53,8)	0 (0)
30	30	17,720,440	AG	8	4 (50)	0 (0)	4 (50)	0 (0)	10	4 (40)	0 (0)	6 (60)	0 (0)
30	30	17,722,040	AT	8	4 (50)	0 (0)	0 (0)	4 (50)	27	17 (63)	0 (0)	0 (0)	10 (37)
30	30	17,722,041	CT	8	0 (0)	4 (50)	0 (0)	4 (50)	27	0 (0)	17 (63)	0 (0)	10 (37)
30	30	17,723,509	GT	6	0 (0)	0 (0)	3 (50)	3 (50)	16	0 (0)	0 (0)	9 (56,2)	7 (43,8)
30	30	17,730,597	CT	4	0 (0)	2 (50)	0 (0)	2 (50)	29	0 (0)	12 (41,4)	0 (0)	17 (58,6)
30	30	17,732,252	GT	11	0 (0)	0 (0)	5 (45,5)	6 (54,5)	13	0 (0)	0 (0)	6 (46,2)	7 (53,8)
30	30	17,735,915	AC	11	6 (54,5)	5 (45,5)	0 (0)	0 (0)	142	65 (45,8)	77 (54,2)	0 (0)	0 (0)
30	30	17,740,620	CG	13	0 (0)	6 (46,2)	7 (53,8)	0 (0)	96	0 (0)	47,04 (49)	48,96 (51)	0 (0)
30	30	17,741,952	AG	11	6 (54,5)	0 (0)	5 (45,5)	0 (0)	37	18 (48,6)	0 (0)	19 (51,4)	0 (0)
30	30	17,749,897	AT	15	8 (53,3)	0 (0)	0 (0)	7 (46,7)	64	23 (36)	0 (0)	0 (0)	41 (64)
30	30	17,757,584	AT	9	4 (44,4)	0 (0)	1 (11,2)	4 (44,4)	160	73 (45,6)	0 (0)	0 (0)	87 (54,4)
30	30	17,761,707	AG	12	6 (50)	0 (0)	6 (50)	0 (0)	28	12 (42,9)	0 (0)	16 (57,1)	0 (0)
30	30	17,769,534	CT	19	0 (0)	9 (47,4)	0 (0)	10 (52,6)	65	0 (0)	32 (49,2)	0 (0)	33 (50,8)
30	30	17,776,973	CT	22	0 (0)	10 (45,5)	0 (0)	12 (54,5)	107	0 (0)	66 (61,7)	0 (0)	41 (38,3)
30	30	17,779,125	CT	10	0 (0)	5 (50)	0 (0)	5 (50)	37	0 (0)	19 (51,4)	0 (0)	18 (48,6)
30	30	17,784,677	CG	19	0 (0)	10 (52,6)	9 (47,4)	0 (0)	121	0 (0)	72 (59,5)	49 (40,5)	0 (0)
30	30	17,785,788	AG	9	4 (44,4)	0 (0)	5 (55,6)	0 (0)	176	81 (46)	0 (0)	91 (51,7)	4 (2,3)
30	30	17,789,362	CT	14	0 (0)	8 (57,1)	0 (0)	6 (42,9)	89	0 (0)	52 (58,4)	0 (0)	37 (41,6)

30	30	17,790,340	AT	14	7 (50)	0 (0)	0 (0)	7 (50)	209	109 (51,7)	0 (0)	0 (0)	101 (48,3)
30	30	17,791,231	CT	13	0 (0)	6 (46,2)	0 (0)	7 (53,8)	19	0 (0)	8 (42,1)	0 (0)	11 (57,9)
30	30	17,798,732	CG	12	0 (0)	7 (58,3)	5 (41,7)	0 (0)	46	0 (0)	17 (37)	29 (63)	0 (0)
30	30	17,811,528	AG	7	3 (42,9)	0 (0)	4 (57,1)	0 (0)	5	2 (40)	0 (0)	3 (60)	0 (0)
30	30	17,812,058	GT	11	0 (0)	0 (0)	5 (45,5)	6 (54,5)	24	0 (0)	0 (0)	15 (62,5)	9 (37,5)