Table S1: Statistics of the ONT datasets.

	DH-Pahang		
	Raw reads PromethION	Longest reads	Filtlong reads
Cumulative size	92,749,841,432	13,500,017,777	13,500,035,543
# of reads	5,185,398	175,937	273,413
Coverage	206x	30x	30x
N50 (bp)	31,640	74,681	52,825

Table S2: DH-Pahang ONT assembly statistics.

	SMARTDeNovo			Redbean		
Subset of reads used	All reads	Longest	Filtlong	All reads	Longest	Filtlong
Cumulative size	482,349,453	465,668,591	431,317,546	2,021,646,269	802,627,210	606,906,240
# contigs	121	183	783	43,296	13,785	10,307
N50 (L50)	19,507,988 (9)	5,667,738 (22)	977,146 (111)	75,016 (6,675)	93,433 (1,502)	92,564 (989)
N90 (L90)	2,709,408 (28)	1,176,439 (92)	230,199 (490)	21,284 (26,326)	25,463 (7,959)	25,353 (6,103)

	Flye			NECAT
Subset of reads used	All reads	Longest	Filtlong	All reads
Cumulative size	470,287,666	471,731,899	439,685,078	485,698,222
# contigs	449	318	953	175
N50 (L50)	15,404,016 (12)	12,916,122 (13)	1,521,384 (73)	32,031,704 (7)
N90 (L90)	2,621,850 (40)	3,315,873 (37)	298,601 (333)	5,650,309 (17)

Table S3: DH-Pahang bionano dataset

	DLE-1 Molecule statistics	BspQI Molecule statistics
Total number of molecules	2,151,406	2,897,832
Total length (Mbp)	209,178.556	398,318,111
Average length (kbp)	97.229	137.109
Molecule N50 (kbp)	129.750	220.875
Label density (/100kb)	13.709	10.502
Number of Flow cell	2	1

Table S4: DH-Pahang bionano genome map

	DLE-1 genome map	BspQI genome map
Genome map number	24	71
Total Genome Map Length (Mbp)	469.764	474.019
Genome Map N50 (Mbp)	35.022	16.002

Table S5: DH-Pahang hybrid scaffolding and polishing

	nanopore contigs polished	Hybrid scaffolds	contigs not scaffolded	final hybrid scaffolds (hybrid scaffolds + contigs not scaffolded)	scaffolds after negative gap resolution and polishing
number	124	16	80	96	97
N50 (L50)	32,091,274 (7)	39,508,388 (6)	249,463 (18)	39,508,388 (6)	39,373,400 (6)
N90 (L90)	5,668,018 (17)	21,536,064 (12)	87,718 (59)	21,536,064 (12)	21,536,112 (12)
maxSize	47,719,325	47,719,325	673,878	47,719,325	47,719,527
Assembly size	485,318,484	471,709,278	14,435,609	486,144,887	484,747,212
% of N	0%	0.18%	0%	0.17%	0.14%

Table S6: Contigs details before and after negative gap resolution. Gaps of 100bp are gaps of unknown length generated by the anchoring of contigs using the genetic map. Gaps of 13bp are gaps of unknown size generated by the BioNano pipeline.

DH-Pahang chromosome	# corresponding NECAT contigs	# hybrid scaffolds	# contigs after negative gap resolution	Gaps length (bp)
chr01	7	1	5	163,709 - 13 - 37,440 - 13
chr02	1	1	1	/
chr03	4	2	2	100
chr04	1	1	1	/
chr05	4	1	4	53,161 - 30,868 - 32,283
chr06	1	1	1	/
chr07	5	1	4	13 - 13 - 13
chr08	4	2	4	100 - 46,534 - 324,396
chr09	1	1	1	/
chr10	8	2	2	100
chr11	1	1	1	/

Table S7: **Marker number per scaffold and linkage group**

scaffold	LG01-04	LG02	LG03	LG05	LG06	LG07	LG08	LG09	LG10	LG11
Pahang_Scaffold_1	0	0	0	1	0	1	0	2089	0	0
Pahang_Scaffold_2	0	0	0	1874	0	1	0	0	1	0
Pahang_Scaffold_3	2191	1	0	0	0	0	0	1	0	0
Pahang_Scaffold_4	0	0	1	0	2190	0	0	1	0	0
Pahang_Scaffold_5	1082	0	2	3	0	0	2	1	0	0
Pahang_Scaffold_6	0	0	0	1	0	1687	0	0	1	1
Pahang_Scaffold_7	0	0	0	0	1	0	0	0	1784	0
Pahang_Scaffold_8	0	1483	0	0	0	0	0	0	0	0
Pahang_Scaffold_9	0	0	0	1	0	0	1	1	0	1500
Pahang_Scaffold_10	0	0	0	1	0	0	1283	0	0	0
Pahang_Scaffold_11	0	0	1027	1	0	0	0	0	0	0
Pahang_Scaffold_12	0	0	0	0	0	0	1276	0	0	0
Pahang_Scaffold_13	0	0	850	0	0	0	1	0	1	0
Pahang_Scaffold_14	0	0	0	1	0	0	0	0	173	0
Pahang_Scaffold_59	1	0	0	0	0	0	0	0	0	0

Table S8: TE classes proportions in *Musa acuminata* (V2 and V4), *Musa schizocarpa* and *Musa balbisiana*.

		Musa acuminata V4 (%)	*Musa acuminata* V2 (%)	*Musa schizocarpa* (%)	*Musa balbisiana* (%)
Class I (retrotransposons)					
LTR	Copia	11,079	8,965	13,766	12,432
	Gypsy	5,986	4,668	5,848	5,137
	no cat	17,788	12,743	19,767	16,547
DIRS	RYX	6,323	3,252	6,094	4,157
PLE	Penelope	0,003	0,003	0,003	0,004
LINE	RIL/RIX	3,492	2,741	3,478	2,941
SINE	RSX	0,005	0,006	0,009	0,004
Large Retro-transposon Derivatives	RXX	2,678	1,347	2,590	1,597
Class II (DNA transposons)-Subclass 1					
TIR	DTX	0,163	0,172	0,180	0,185
hAT	DTA	0,506	0,538	0,525	0,637
Class II (DNA transposons)-Subclass 2					
Helitron	DHH/DHX	2,292	2,175	1,737	2,152
Maverick	DMX	0,005	0,006	0,007	0,006
MITE (miniature inverted repeat transposable elements)	DXX	0,029	0,028	0,023	0,027
No categories		0,722	0,549	1,097	0,762
simple repeat		1,549	1,190	1,221	2,767
	TOTAL	52,623	38,383	56,345	49,353

Table S9: Proportion of new annotated genes in each *Musa acuminata* chromosome of the V4 assembly.

	Nb of annotated genes	Nb of tandemly duplicated genes	Nb of new annotated genes	Nb of new tandemly duplicated genes
chr01	2,757	397 (14.40%)	369 (13.38%)	173 (46,88%)
chr02	2,680	300 (11.19%)	232 (8.66%)	88 (37,93%)
chr03	3,533	314 (8.89%)	235 (6.65%)	85 (36,17%)
chr04	4,254	343 (8.06%)	258 (6.06%)	66 (25,58%)
chr05	3,345	263 (7.86%)	251 (7.50%)	73 (29,08%)
chr06	4,076	349 (8.56%)	283 (6.94%)	96 (33,92%)
chr07	3,080	324 (10.52%)	190 (6.17%)	83 (43,68%)
chr08	3,604	299 (8.30%)	221 (6.13%)	67 (30,32%)
chr09	3,342	342 (10.23%)	243 (7.27%)	84 (34,57%)
chr10	3,552	527 (14.84%)	510 (14.36%)	250 (49,02%)
chr11	2,612	175 (6.70%)	171 (6.55%)	42 (24,56%)
Total	36,835	3,633 (9.86%)	2,963 (8.04%)	1,137 (38.37%)

Table S10: Comparison of 5S ribosomal gene clusters in V2 and V4 Musa assemblies

	Base pair covered in V2 assembly (predicted genes)	Base pair covered in V4 assembly (predicted genes)
chr01	0	59,949 (1,882)
chr02	0	0
chr03	194 (3)	179,282 (4,645)
chr04	0	413 (10)
chr05	529 (21)	640 (19)
chr06	68 (1)	68 (1)
chr07	419 (7)	216 (3)
chr08	4,104 (38)	127,057 (1,135)
chr09	547 (14)	80 (1)
chr10	116 (6)	0
chr11	0	0
Un	1,605 (40)	-
Total	7,582 (130)	367,705 (7,696)

Table S11: Comparison of NLR clusters between the V2 and V4 assemblies (based on NLR-Annotator predictions)

Assembly version	Chromosome	Cluster start coordinate	Cluster end coordinate	Size (bp)	Nb. Undetermined nucleotides (N)	Nb. NLR loci detected
V2	chr03	27,854,779	27,992,717	137,938	5,255	14
V2	chr03	31,895,636	32,022,409	126,773	10,472	17
V2	chr07	29,765,171	29,842,567	77,396	4,269	6
V2	chr10	22,396,059	22,566,146	170,087	13,045	9
V4	chr03	36,566,894	36,731,875	164,981	0	16
V4	chr03	40,651,606	40,784,397	132,791	0	18
V4	chr07	33,870,053	34,018,178	148,125	0	10
V4	chr10	24,960,703	25,188,409	227,706	0	13

Figure S1: gDNA extraction of DH-Pahang. DNA quality was checked on a 2200 TapeStation automated electrophoresis system (Agilent, CA, USA). Ninety seven % of the DNA fragments have a length >50Kb. (a) before removal of small DNA fragments with Short Read Eliminator XL (Circulomics, MD, USA) (b) after removal of small DNA fragments

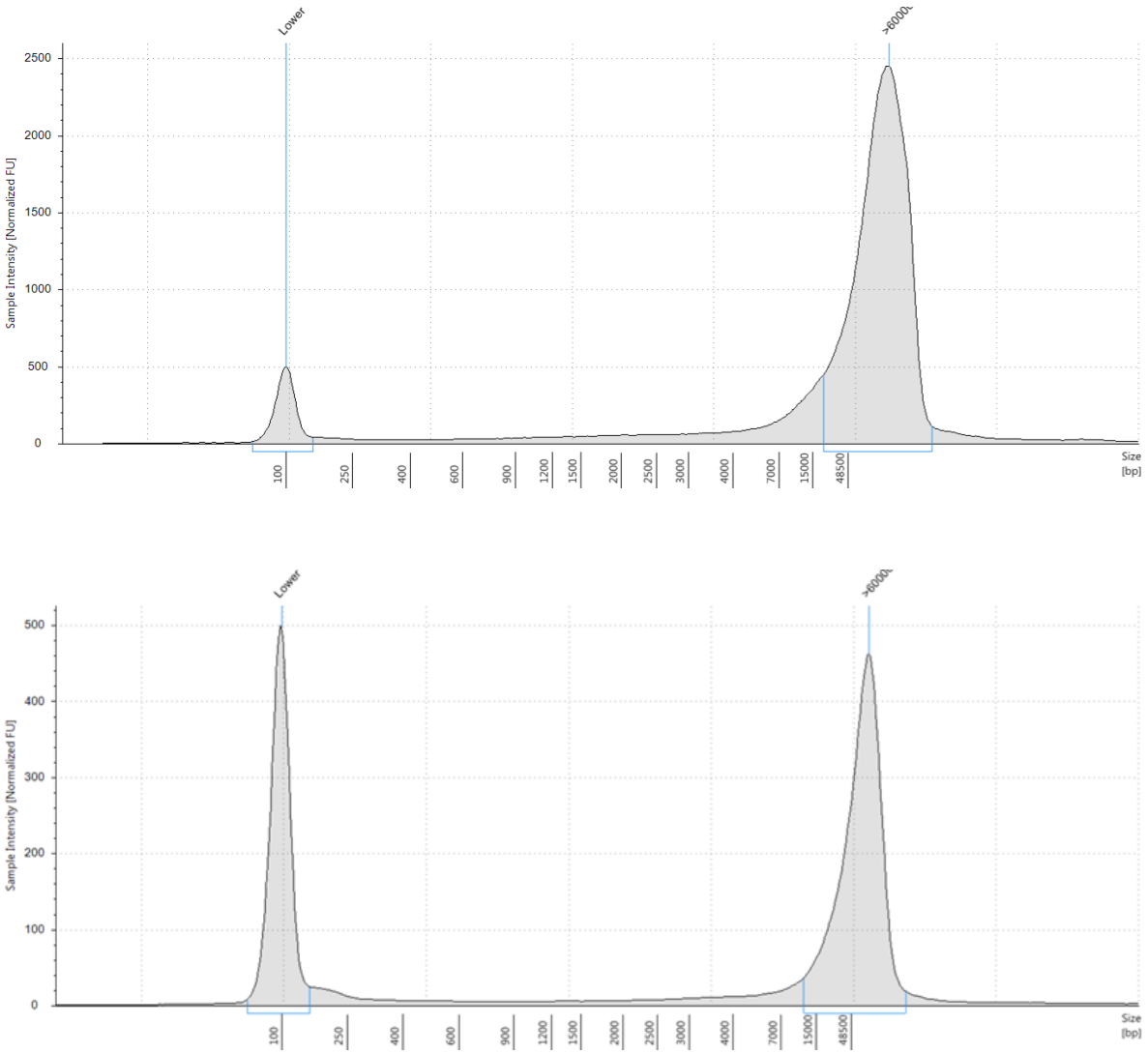


Figure S2: Overview of the nanopore contigs in the V4 assembly. Alignment of the two optical maps (a, red) DLE-1 and (b, red) BspQI with the nanopore contigs (c, blue). The vertical grey lines between the contigs and the optical maps represent the matches between enzymatic cuts from the map and the DNA sequences. The number of contigs of each chromosome is indicated.

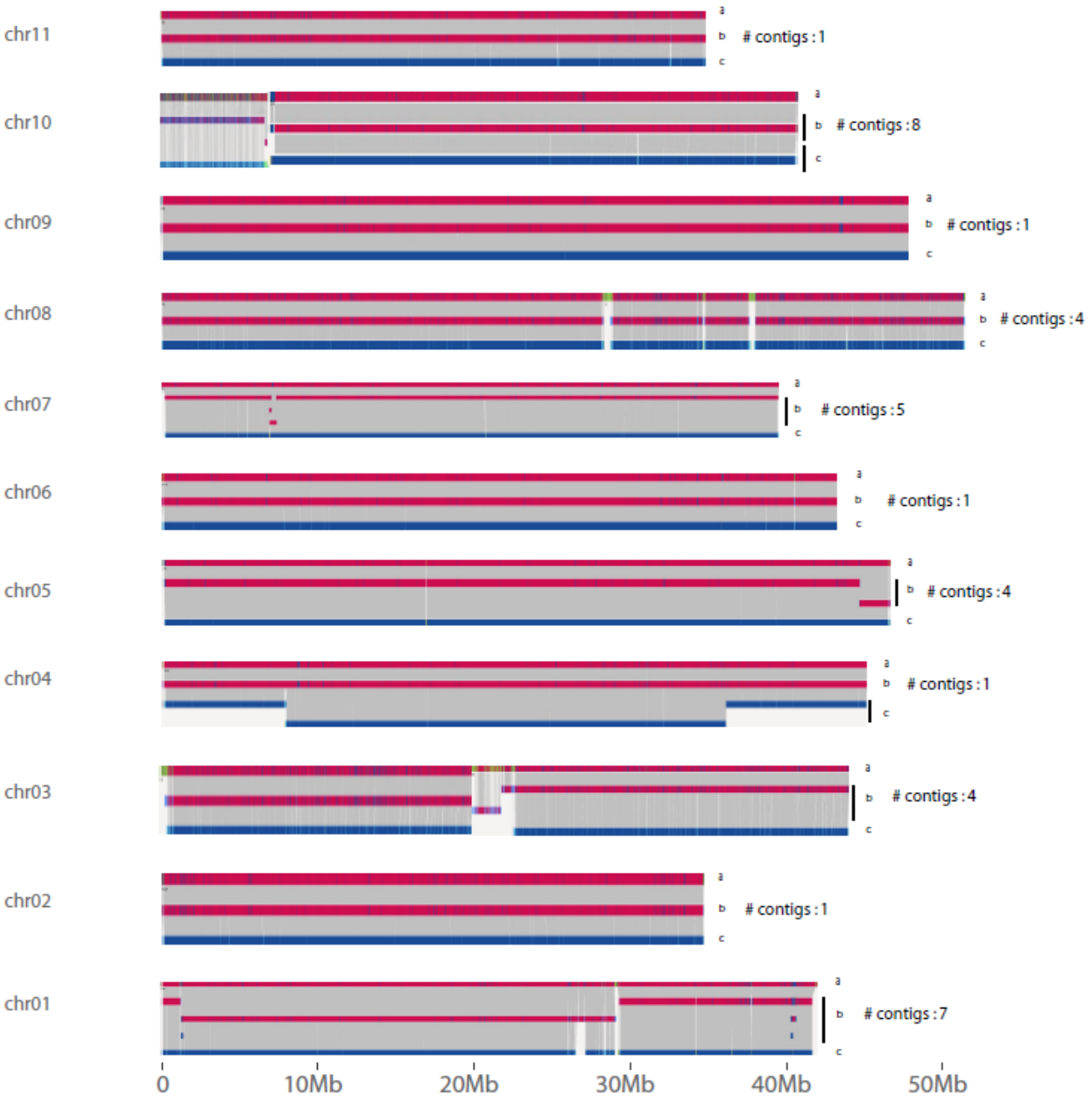


Figure S3: Dot plot showing marker linkage along ordered scaffolds of linkage group 01-04. This figure showed marker linkage of linkage group 01-04 which contained markers from chromosome 01 and 04. Because of chromosomal co-segregation due to reciprocal translocation between chromosome 01 and 04, markers from these chromosomes are linked. This resulted by the linkage of markers from a region of scaffold_3 to a region of scaffold_5. Interpretation of the figure suggested that in fact scaffold_3 corresponded to one chromosome and scaffold_5 corresponded to another chromosome.

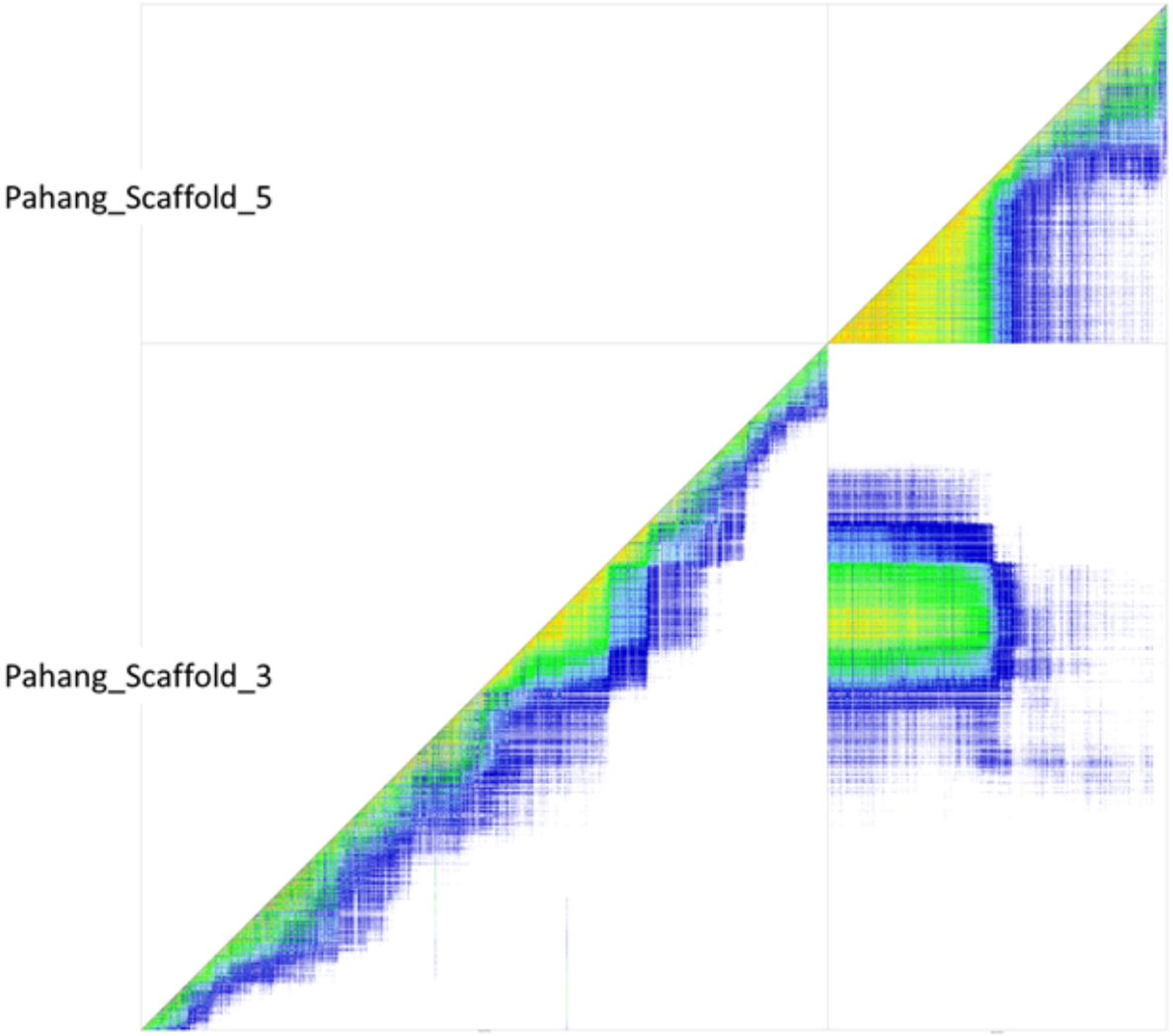


Figure S4: KAT plot of *Musa acuminata* V4 assembly.

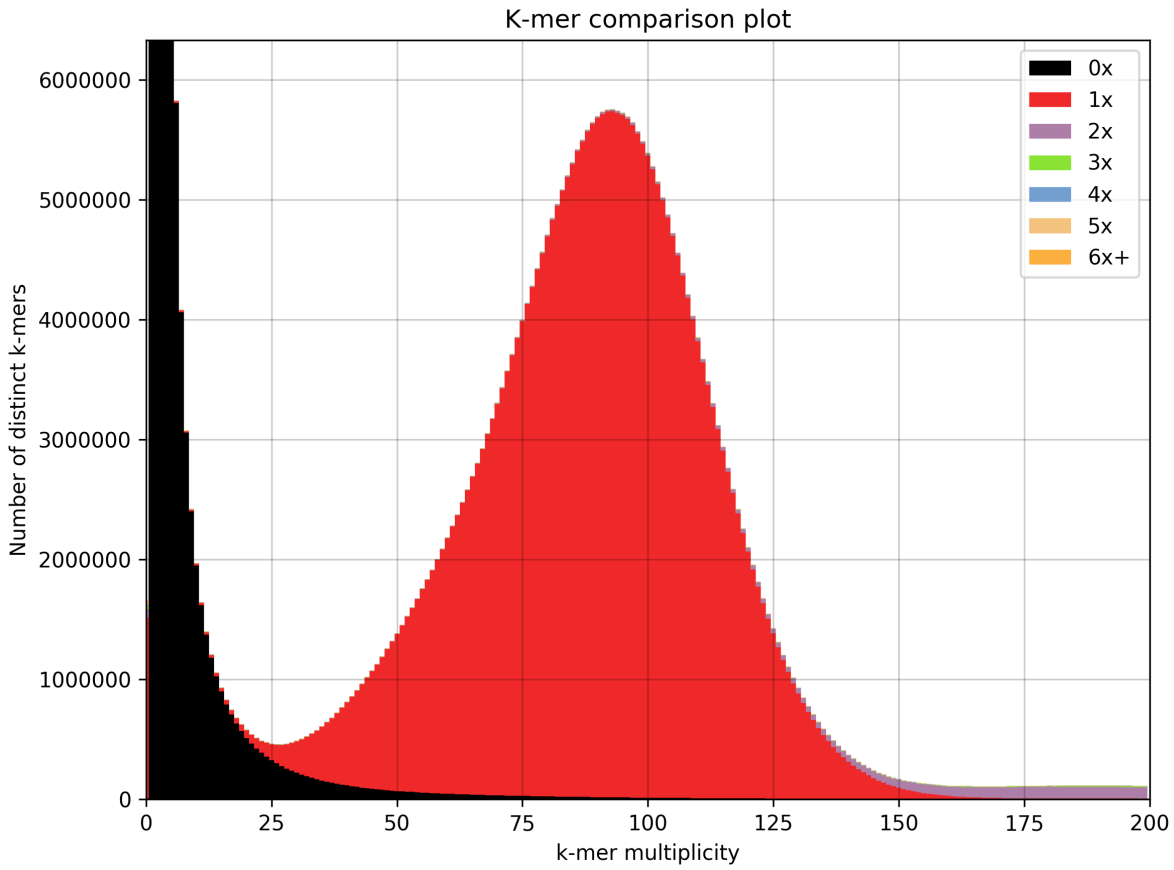


Figure S5: Dot plot of each V4 chromosome against its relative V2 chromosome. The x axis represents the chromosome in V2. The y axis represents the chromosome in V4. The number of the chromosome is printed in brackets.

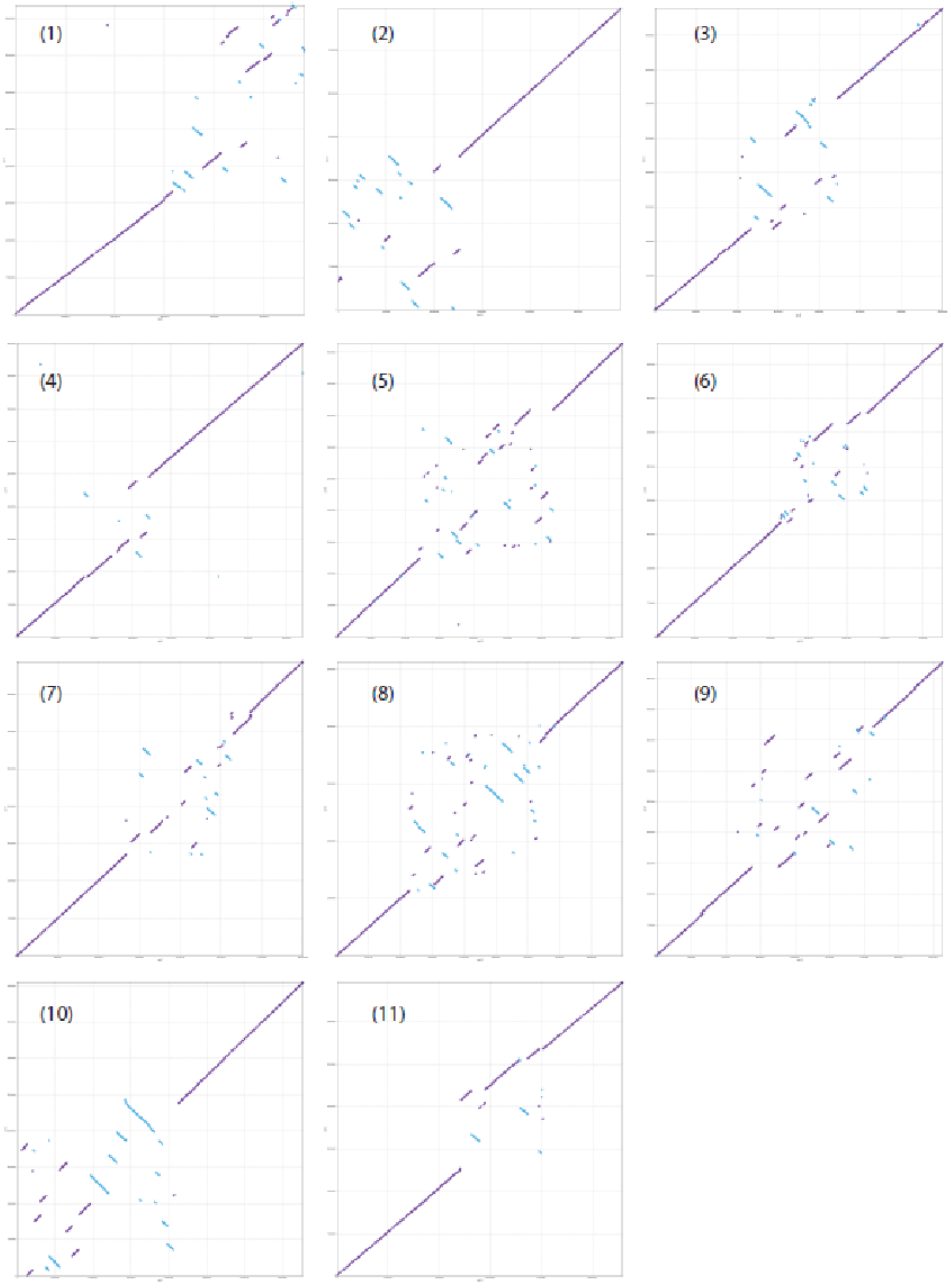


Figure S6: Contig flagged as conflictual with the optical maps. The contigs NGS41 (380,641bp) contains in tandem repetitive elements. The contig was split at the position 299,945b in two contigs of 299,945 et 80,696bp.

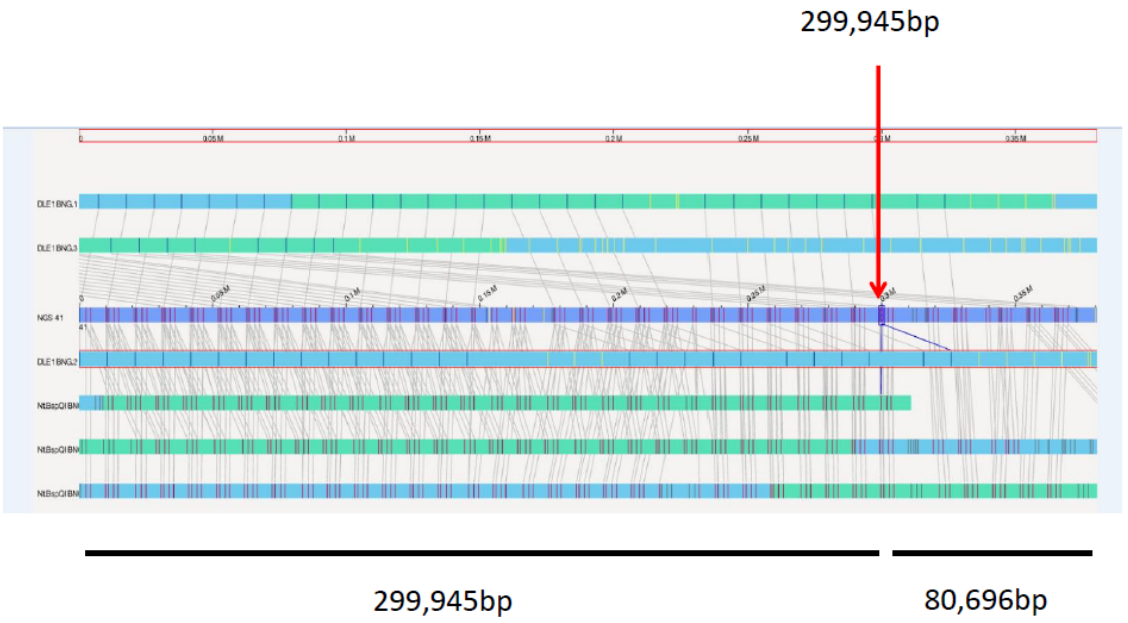


Figure S7: Screenshot of the genome browser focusing on a *Musa acuminata* V4 chromosome 1 region. These new annotated genes in the center of track 1 (Gene Predictions) are included in TDGs cluster and were absent from the *Musa acuminata* V2 annotation.

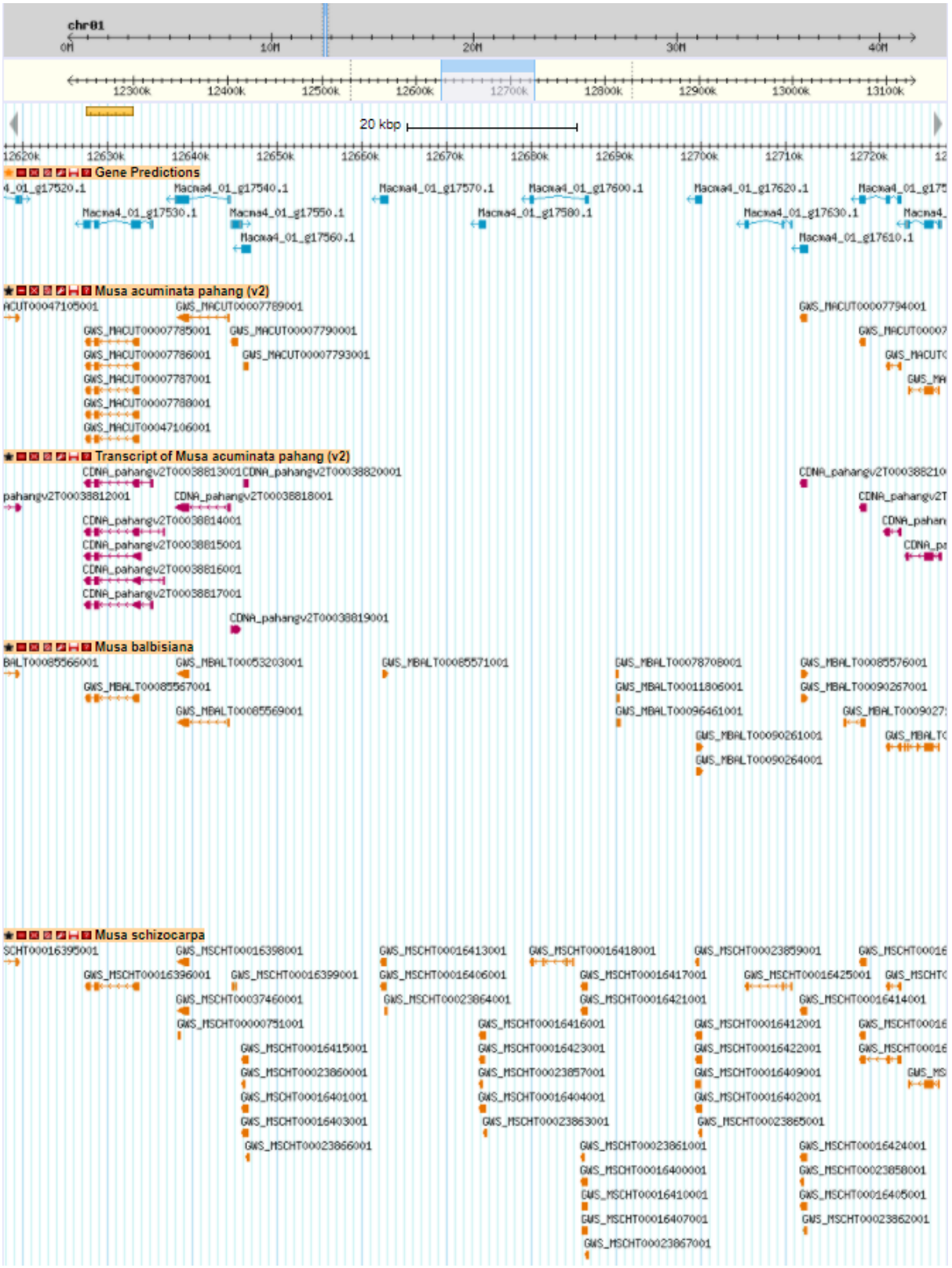


Figure S8: Distribution of the repeats along the V4 chromosomes.

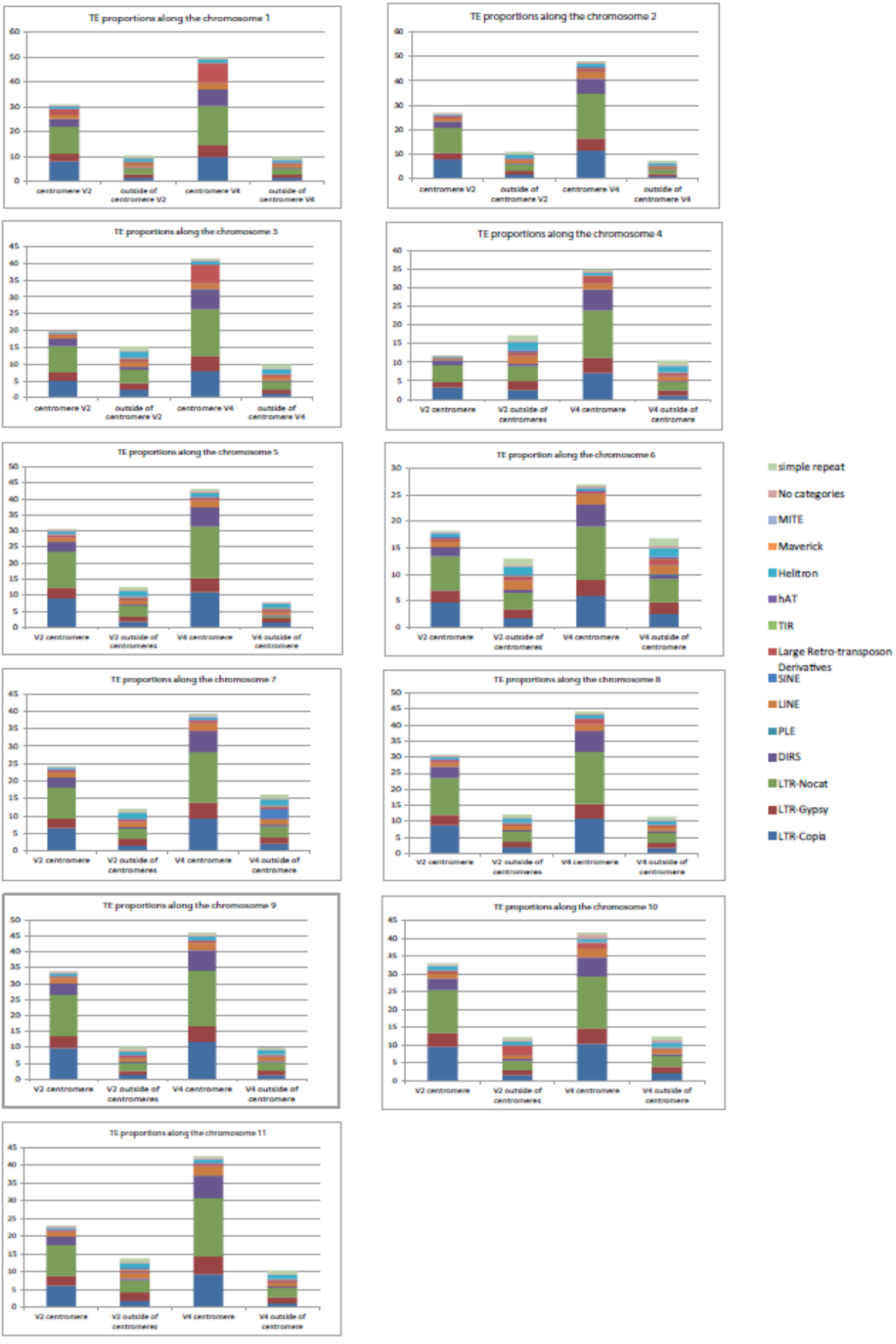
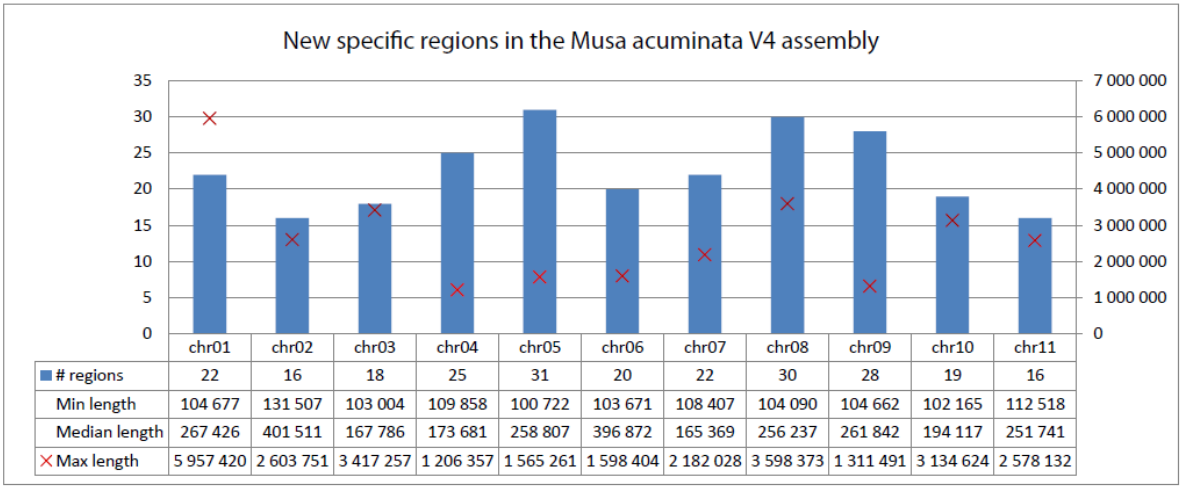


Figure S9: Characterization of specific regions of the *Musa acuminata* V4 assembly.



	chr01	chr02	chr03	chr04	chr05	chr06	chr07	chr08	chr09	chr10	chr11
# regions	22	16	18	25	31	20	22	30	28	19	16
Min length	104 677	131 507	103 004	109 858	100 722	103 671	108 407	104 090	104 662	102 165	112 518
Median length	267 426	401 511	167 786	173 681	258 807	396 872	165 369	256 237	261 842	194 117	251 741
Max length	5 957 420	2 603 751	3 417 257	1 206 357	1 565 261	1 598 404	2 182 028	3 598 373	1 311 491	3 134 624	2 578 132

Figure S10: Composition of specific regions of the *Musa acuminata* V4 assembly.

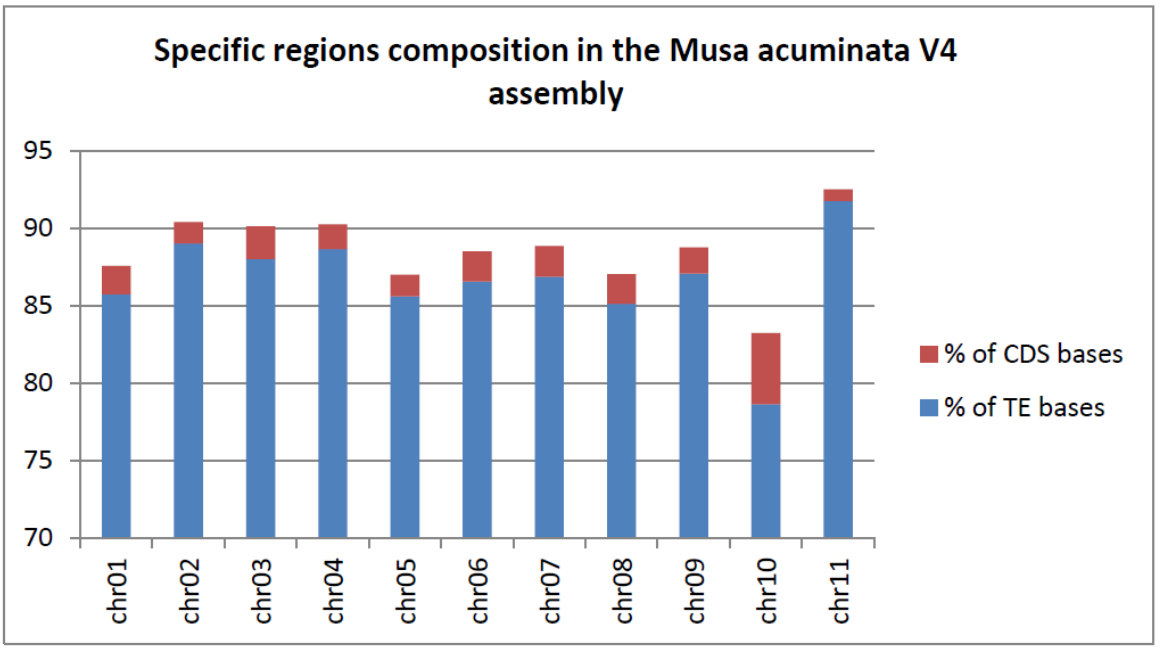


Figure S11: Dot plot of *Musa balbisiana* assembly against *Musa acuminata* V4 assembly.

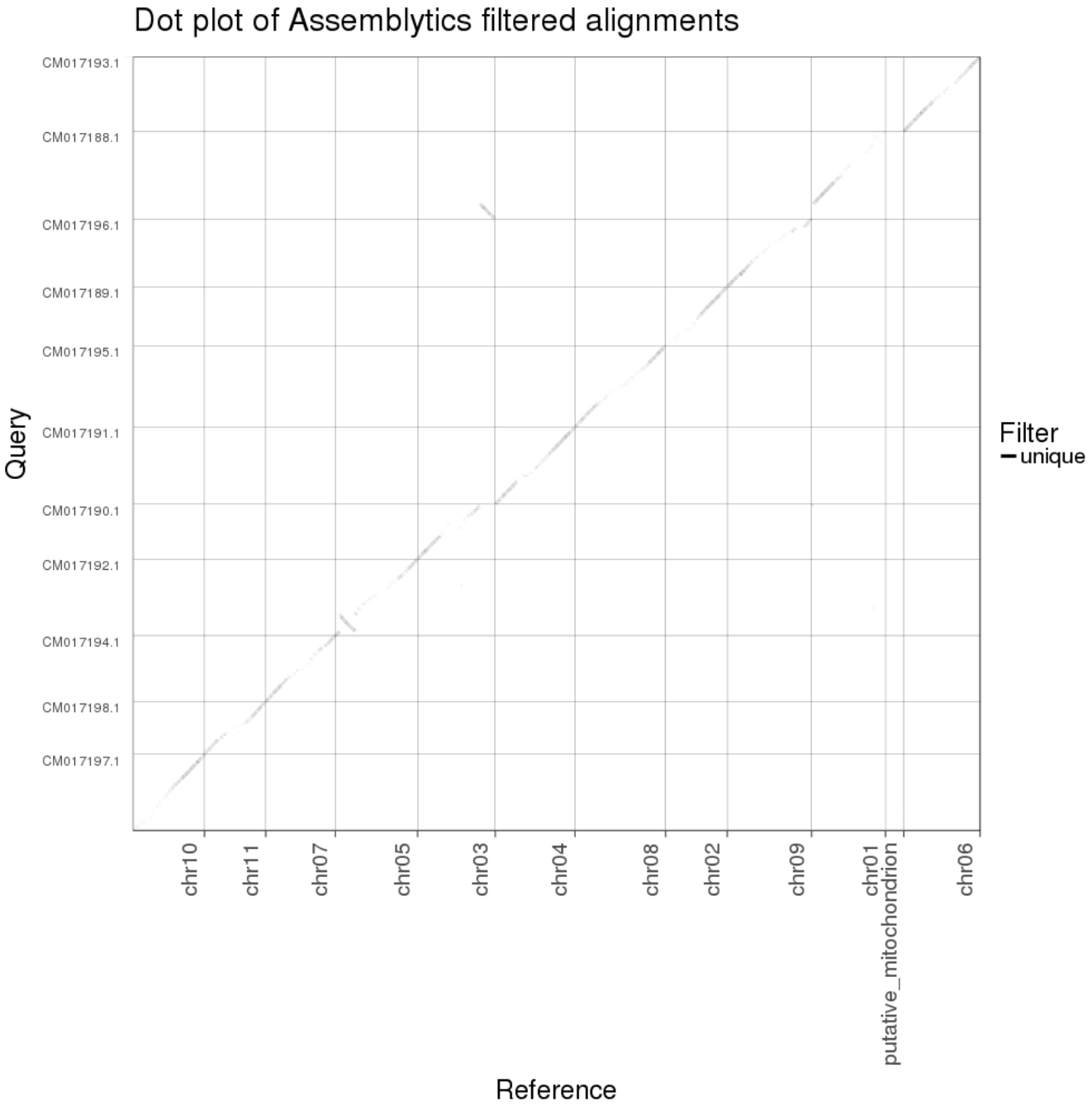


Figure S12: Dot plot of *Musa schizocarpa* assembly against *Musa acuminata* V4 assembly.

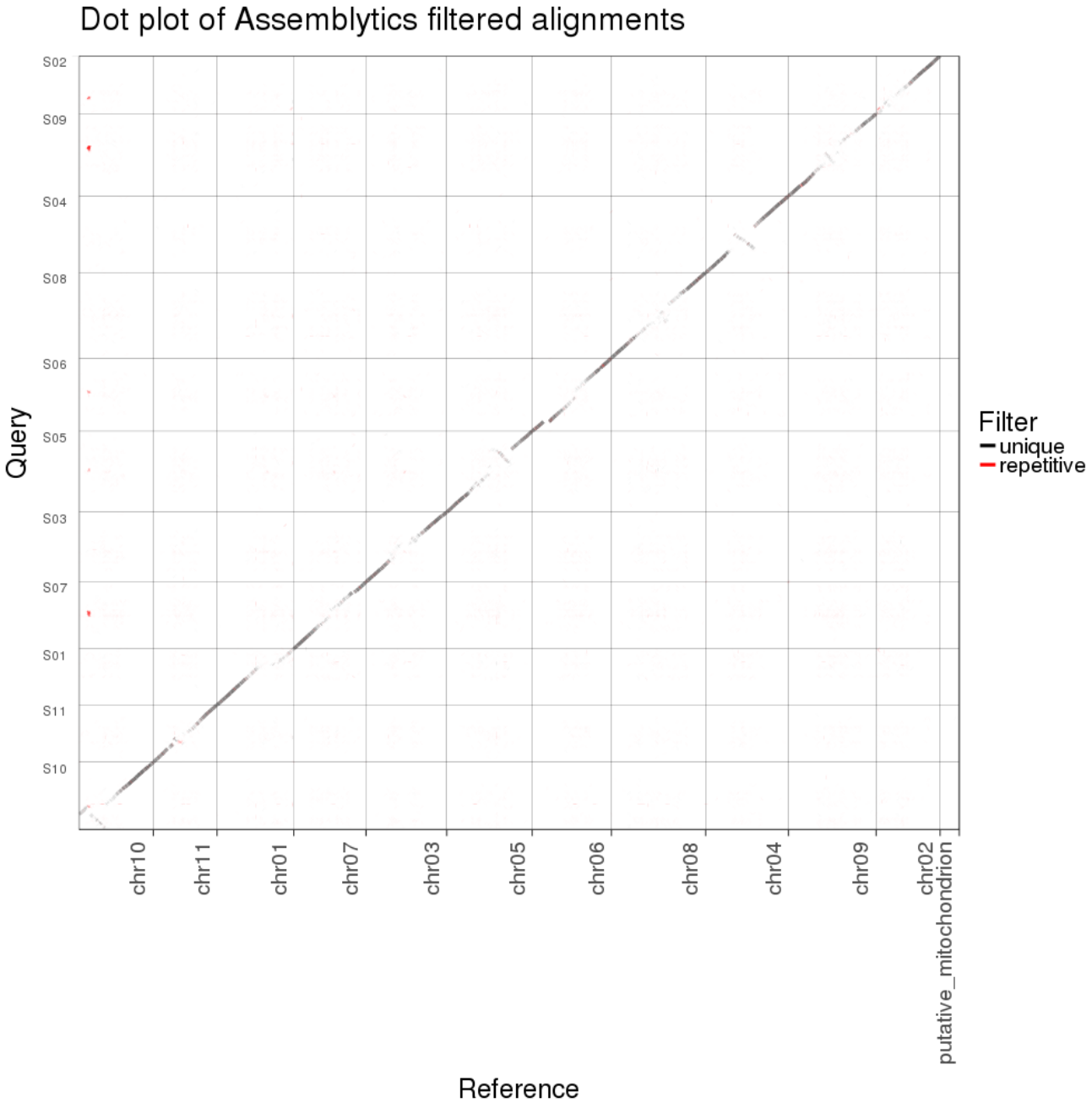


Figure S13: Comparison of the structure of NLR loci clusters between DH-Pahang V2 and V4 assemblies. The four panels represent dot plots of NLR loci clusters on chromosomes 3, 7 and 10 as indicated on top of each panel. The predicted NLR loci for each version are represented on the right side and at the bottom of the dot plots by blue boxes. Red boxes represent regions bearing undetermined nucleotides. Region coordinates are also indicated.

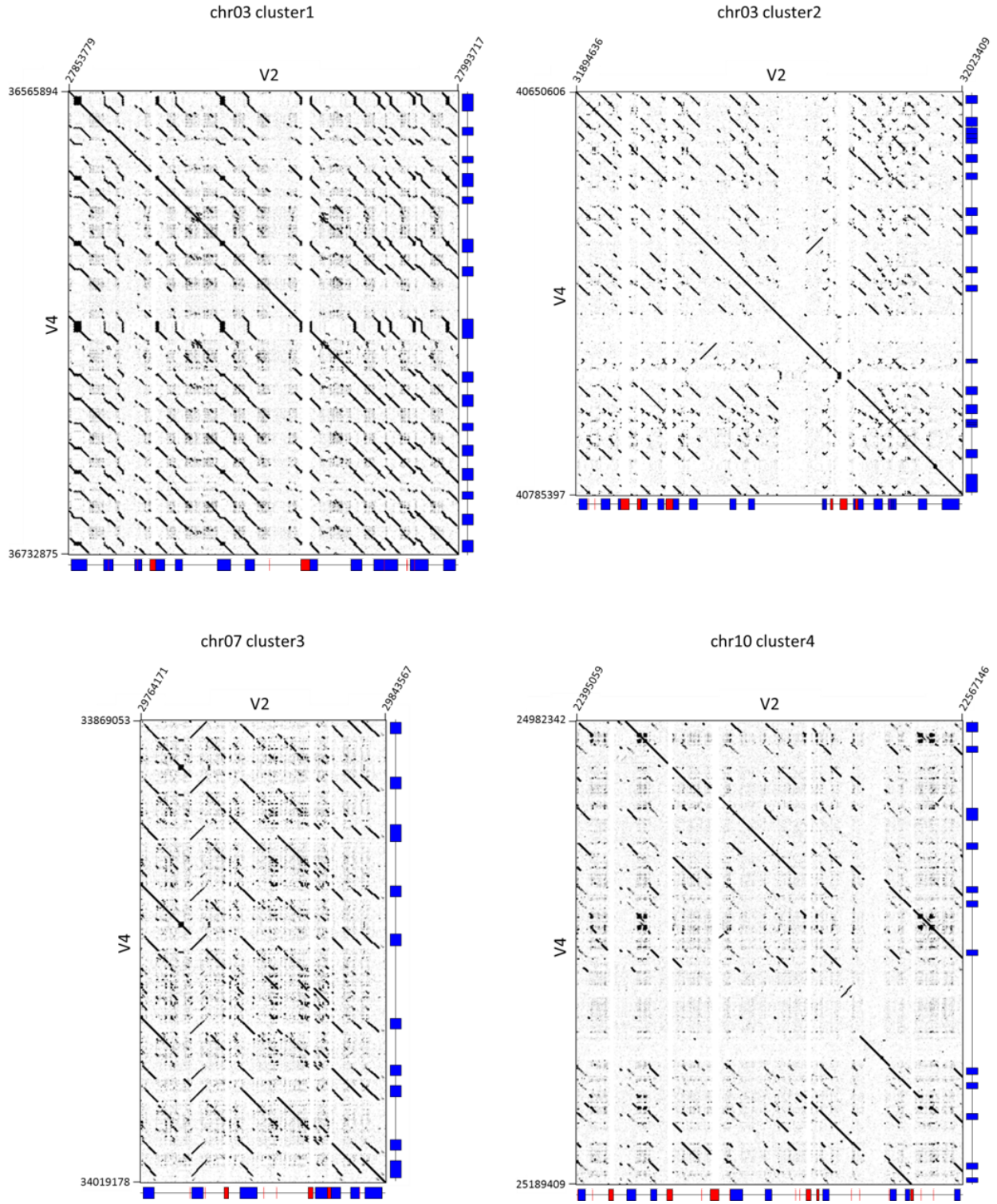


Figure S14: Remaining gaps after negative gap resolution. Example of a remaining sized gap in chromosome 1, located between the position 29,171,567 and 29,335,274. The gap was sized thanks to the BspQI map.