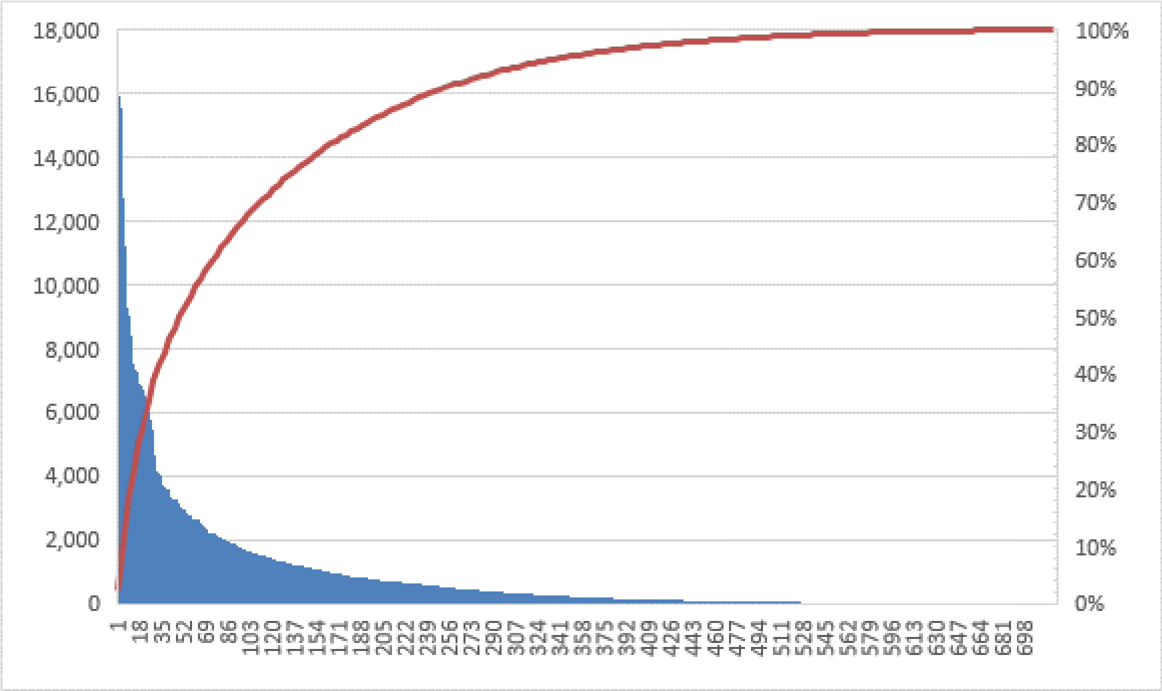
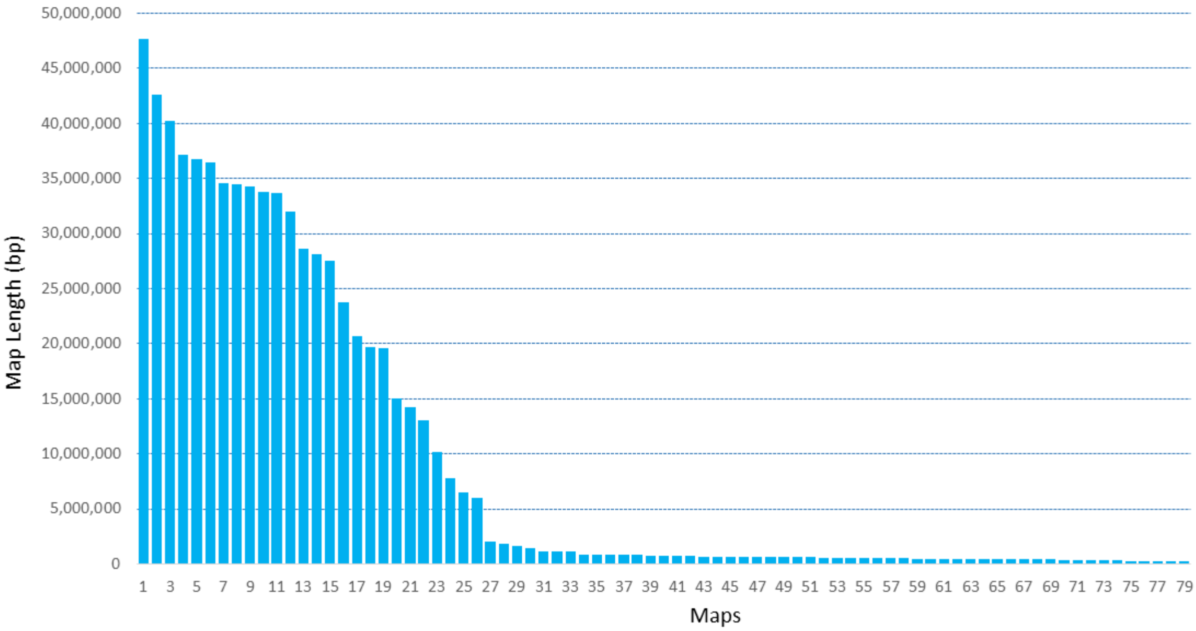
A chromosome-scale assembly of the sorghum genome using nanopore sequencing and optical mapping

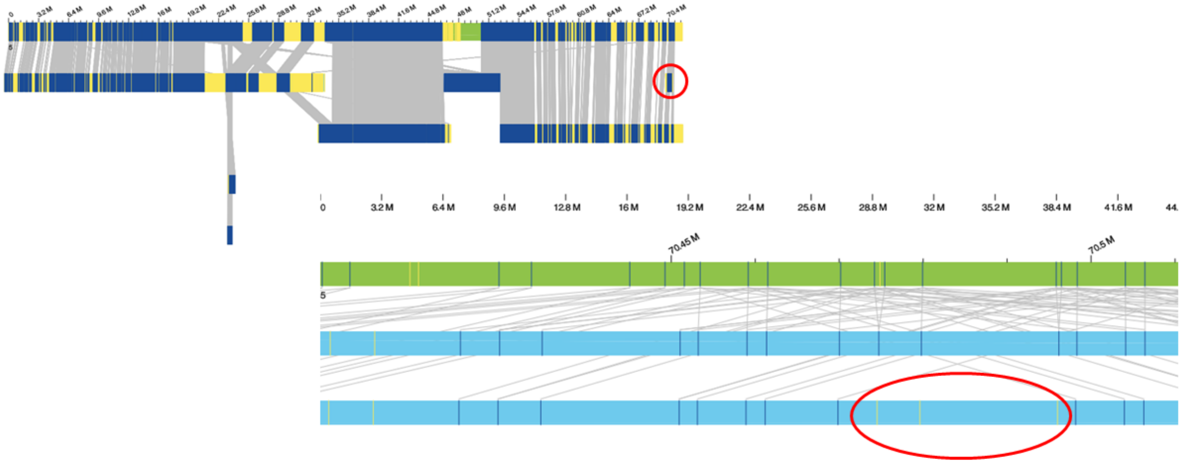
Deschamps et al.



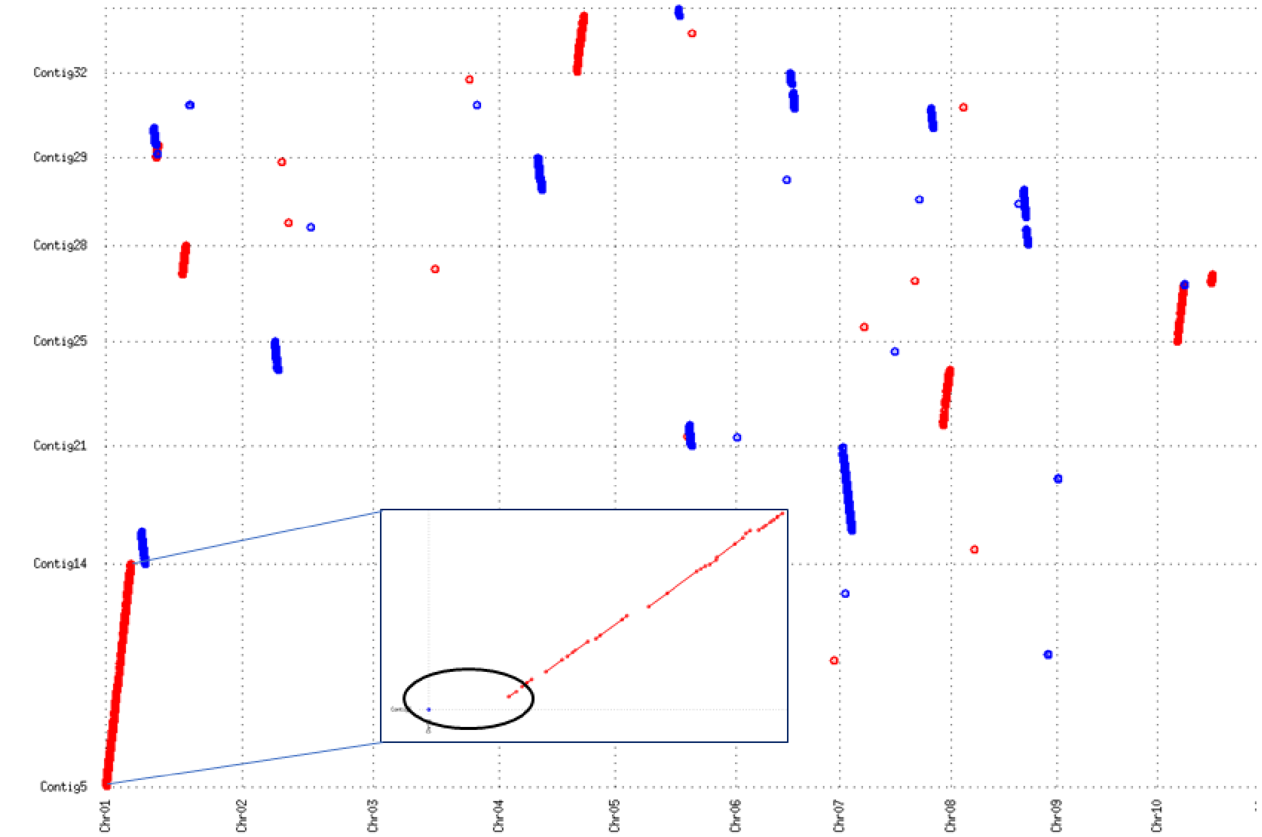
Supplementary Figure 1: Length distribution of ONT contigs after two rounds of polishing with Pilon. Contig size distribution is shown in blue, corresponding to the Y-axis on the left. % completion of the total assembly size is marked by a red line and corresponds to the Y-axis on the right.



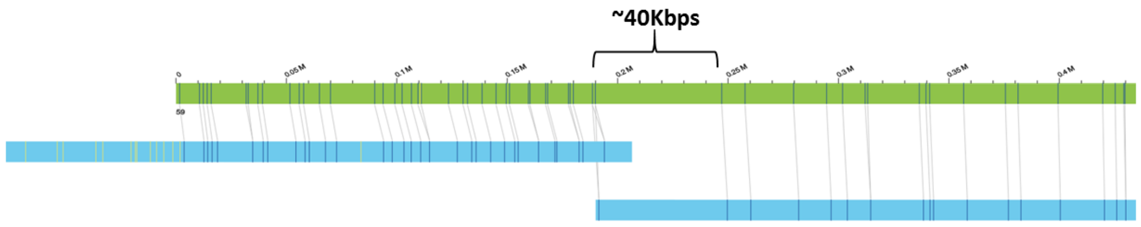
Supplementary Figure 2: Summary of de novo assembly of single molecules into DLS optical maps. A total of 79 maps were created, out of which 32 accounts for 99.5% of the expected assembly size. The assembly N_{50} is 33.77Mbps while the largest map was 47.64Mbps in length.



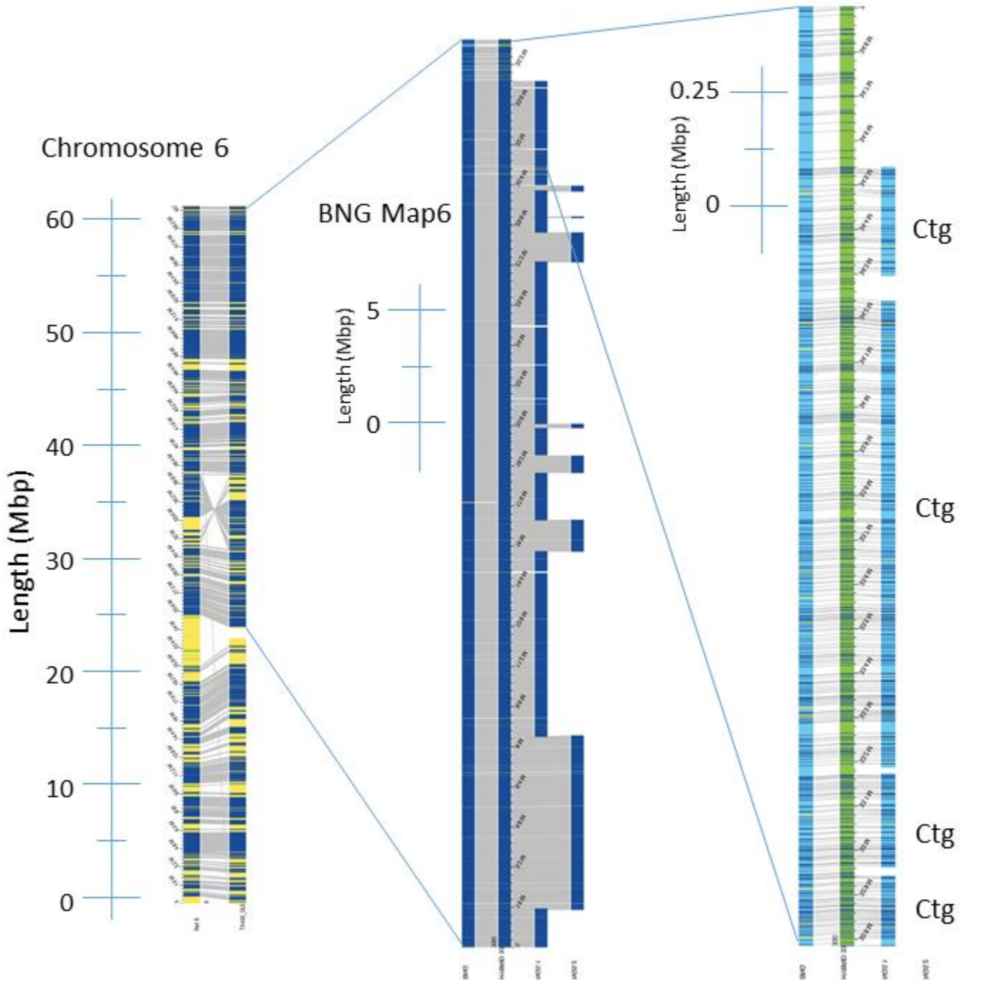
Supplementary Figure 3: Tx430 chromosome 5 and close-up view of overlapping contig. (Upper) Tx430 chromosome 5: v3.0.1 public assembly (top row); DLS optical maps (middle and bottom rows). The overlapping DLS contig is marked by a red circle. (Lower) Close-up view of the overlapping region (details): hybrid scaffold is shown in green. DLS maps are shown in blue. Circles indicate regions of potential heterozygosity on Tx430 chromosome 5 hybrid assembly, corresponding to unmapped DLE-1 motifs, shown in yellow on the DLS maps.



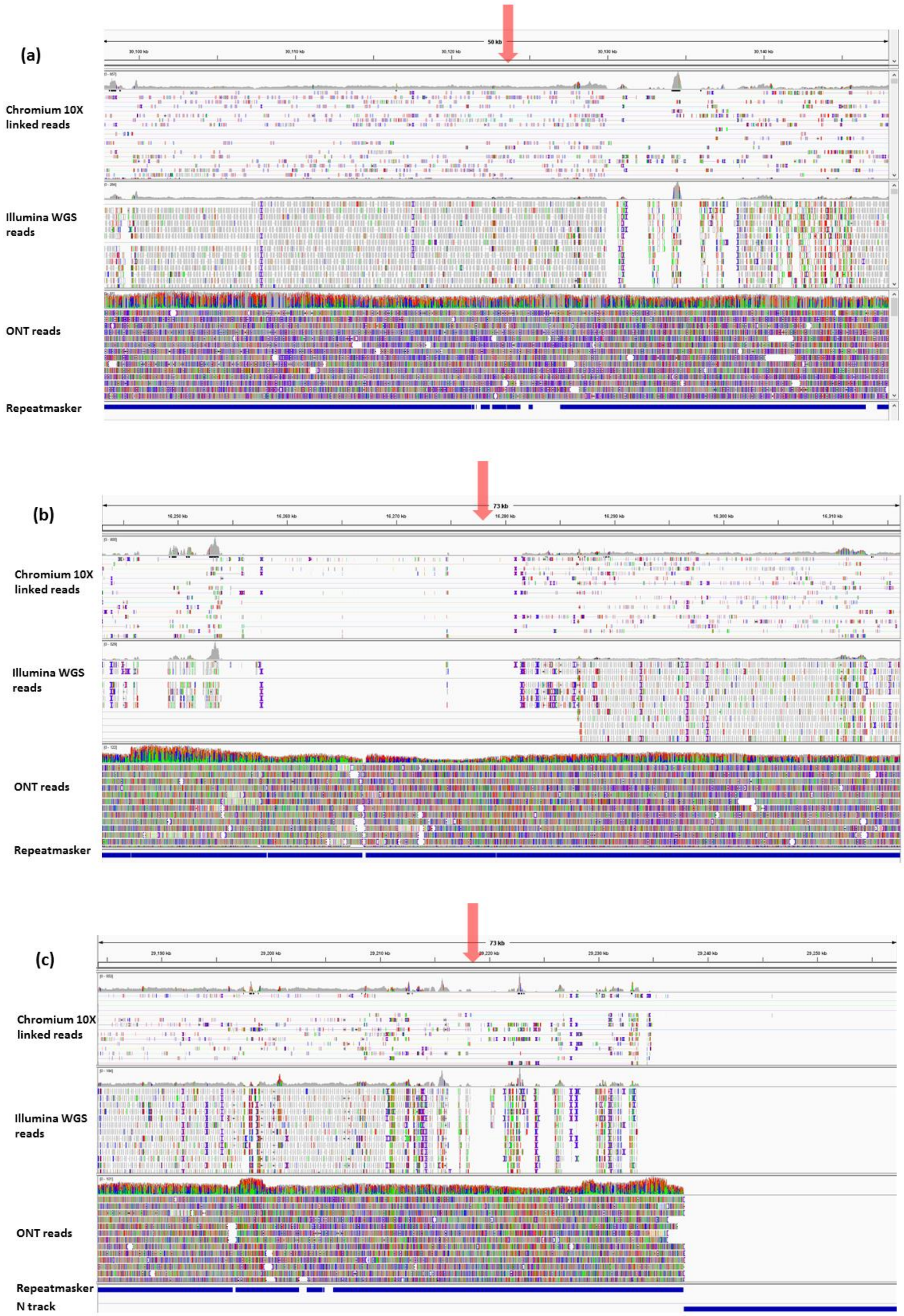
Supplementary Figure 4: NUCmer v3.1 alignments of selected chimeric ONT contigs to the v3.0.1 reference assembly. A subset of all ONT contigs were split into smaller contigs by the Bionano software during hybrid assembly generation. Locations of the splits and the subsequent location of the resulting smaller contigs were confirmed by way of alignment to the v3.0.1 assembly. (X-Axis) v3.0.1 chromosomes; (Y-Axis) a subset of corrected ONT contigs before correction and hybrid assembly generation. Contigs are listed by numbers as determined by the Bionano software. Sequence alignments are shown. The color represents the orientation of the alignment. A close-up version of the split that occurred on the ONT contig mapping to v3.0.1 Chromosome 1 ("Contig5") is shown.



Supplementary Figure 5: Chimeric assembly of Bionano contig maps (details). The ends of two Bionano contig maps (in blue) are merged by the presence of overlapping ONT contig sequences at their respective ends (shown in green), forming a chimeric hybrid map. The chimeric map was corrected by manually deleting the ~40Kbps ONT contig sequence merging the ends of the two Bionano contig maps.



Supplementary Figure 6: Detailed view of a region of Chromosome 6 and corresponding DLS maps. Left: DLS maps aligning to *in-silico* maps of Chromosome 6 from public v3.0.1 assembly; Center: Close-up view of DLS map 6 and mapped ONT contigs; Right: Close-up views of hybrid maps (center – green) generated by merging DLS maps (left – blue) and in-silico maps of ONT contigs (“Ctg”, right – blue). Distances are shown, in Mbps.


(a)
50 kb
30,100 kb
30,110 kb
30,120 kb
30,130 kb
30,140 kb
Chromium 10X linked reads
Illumina WGS reads
ONT reads
Repeatmasker
(b)
73 kb
16,250 kb
16,260 kb
16,270 kb
16,280 kb
16,290 kb
16,300 kb
16,310 kb
Chromium 10X linked reads
Illumina WGS reads
ONT reads
Repeatmasker
(c)
73 kb
29,190 kb
29,200 kb
29,210 kb
29,220 kb
29,230 kb
29,240 kb
29,250 kb
Chromium 10X linked reads
Illumina WGS reads
ONT reads
Repeatmasker
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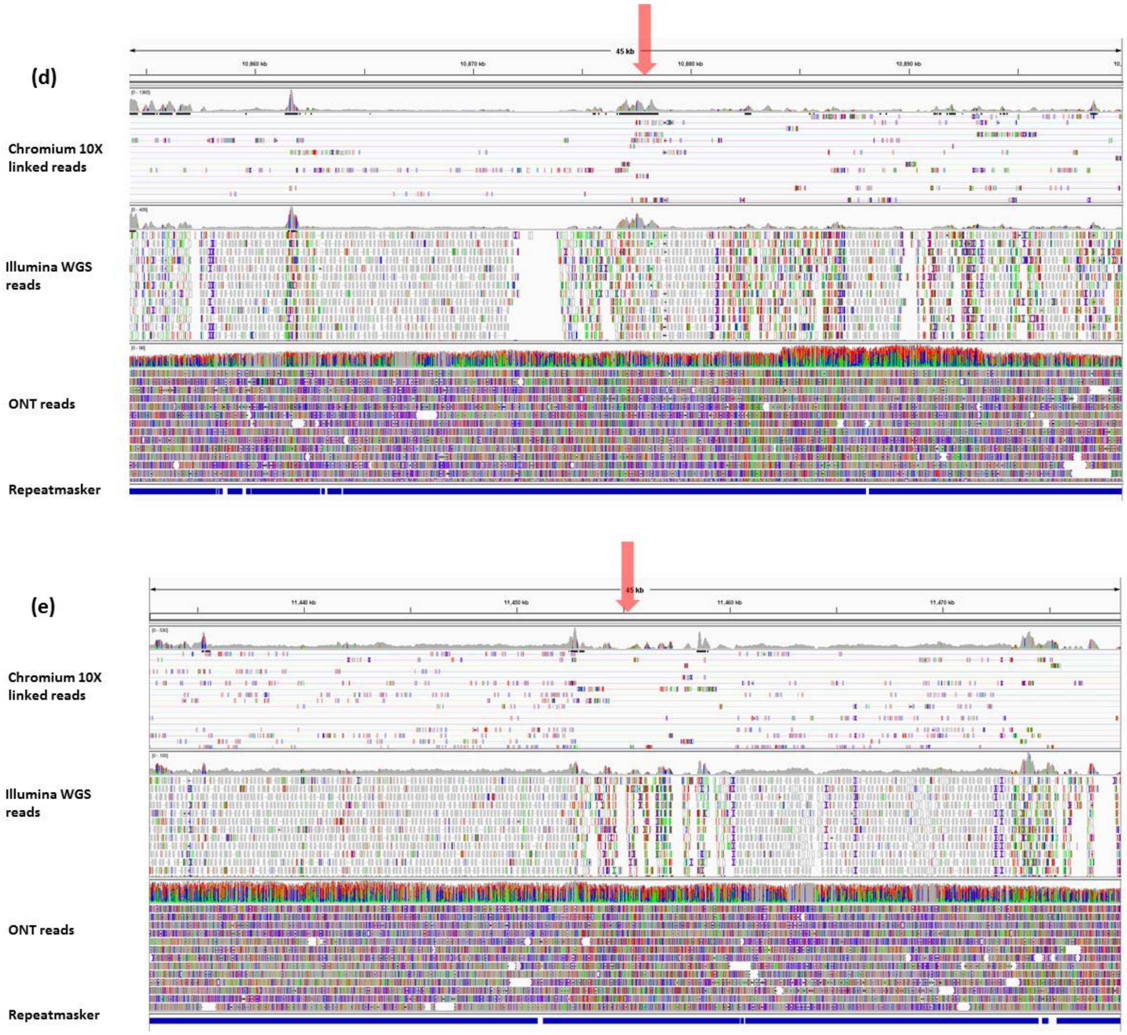
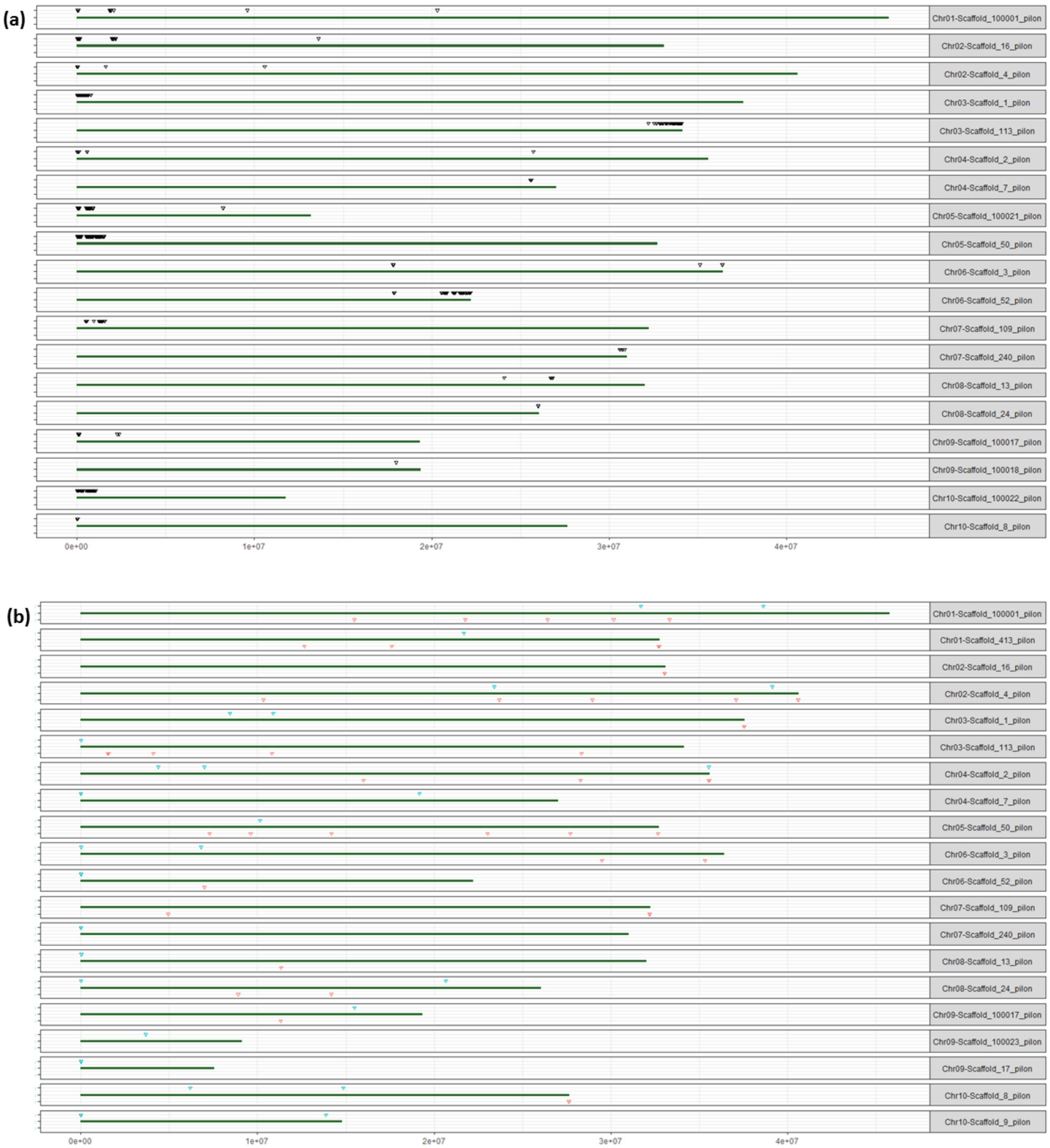
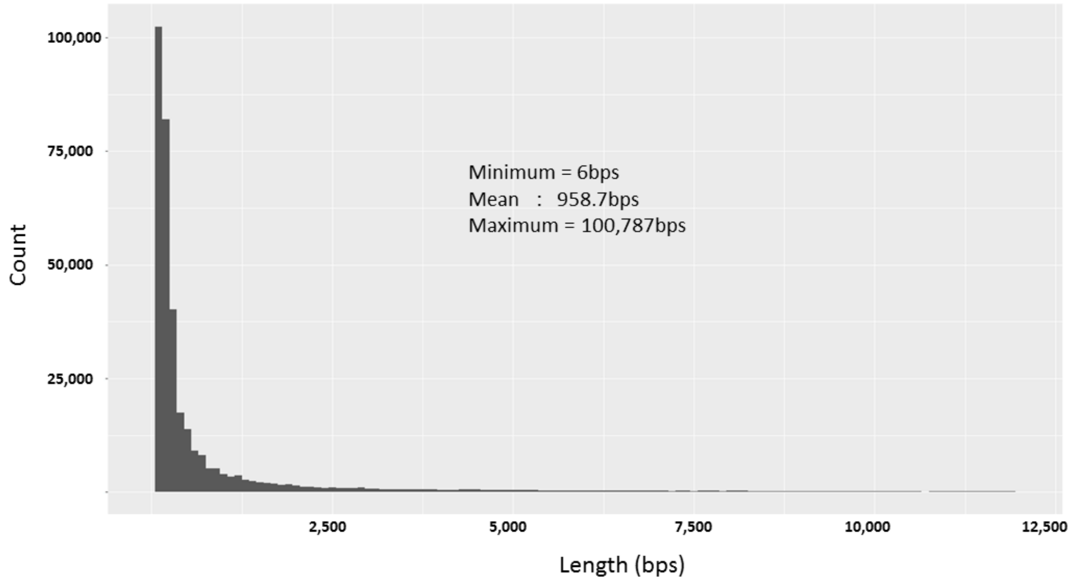


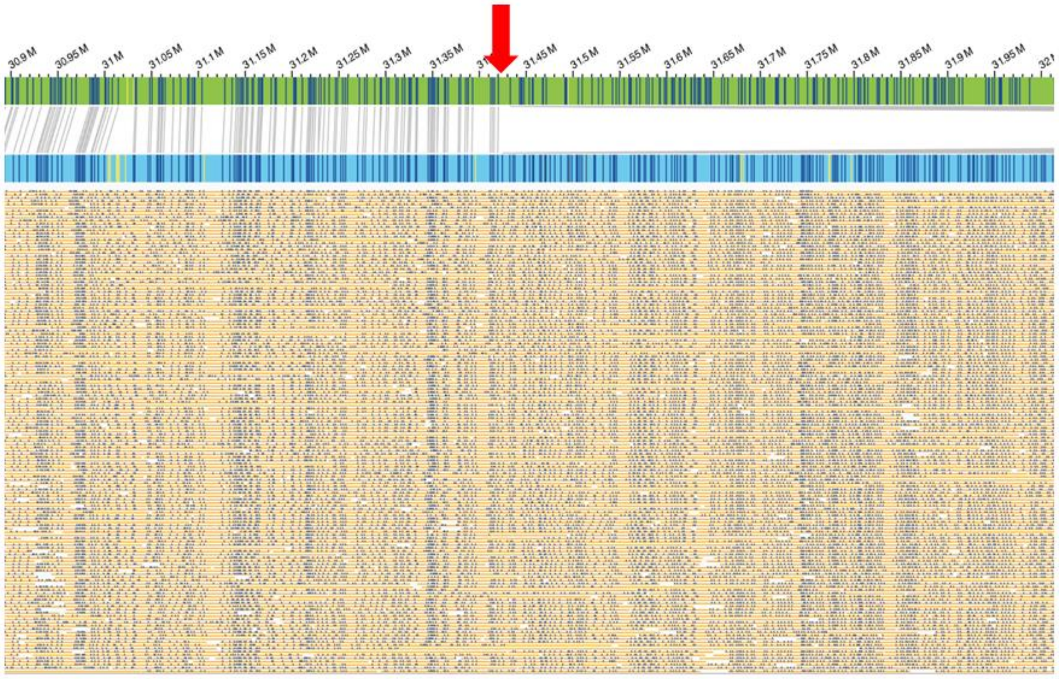
Supplementary Figure 7: Close-up view of the inversion breakpoints located on Chromosome 6, 7 and 9. TX430 Chromium 10X linked reads, Tx430 Illumina whole genome shotgun (WGS) reads, individual Tx430 ONT reads aligning to the breakpoint region, as well as RepeatMasker output and stretches of N's in the hybrid Tx430 scaffolds are shown, from top to bottom. The approximate locations of the inversion's end are marked by an arrow. (a) Chromosome 6; (b) and (c) Chromosome 7; (d) and (e) Chromosome 9.



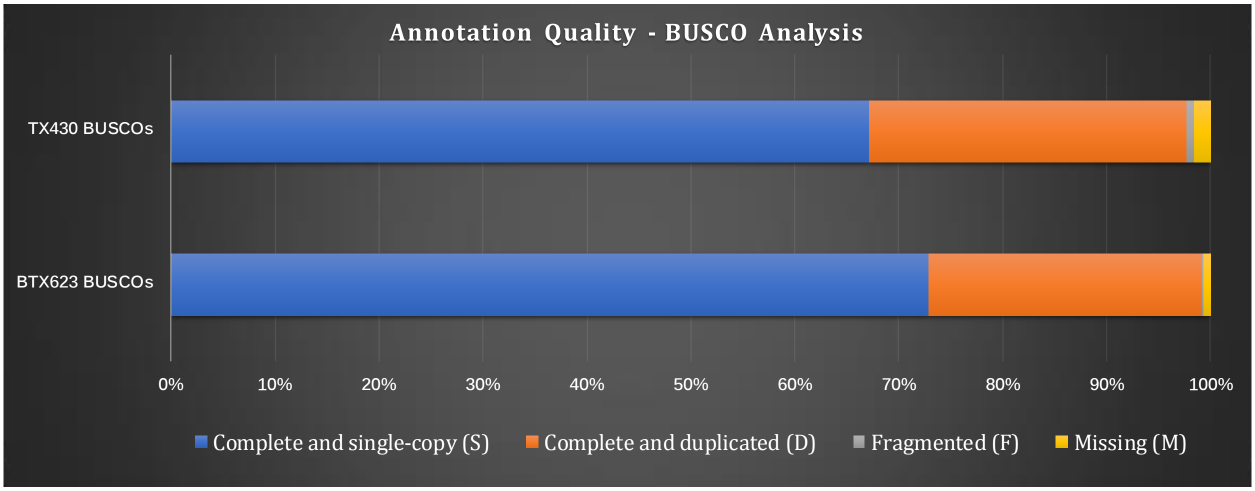
Supplementary Figure 8: detection of centromeric CEN38 and telomeric (CCCTAAA)$_n$ motifs on hybrid scaffolds. (a) Clusters of CEN38 motifs (at least 50 CEN38 hits per clusters; >80% coverage; >80% identity) and their locations are marked by a black triangle on each scaffold. (b) (CCCTAAA) $_n$ motifs (n=4; 28bps perfect match) are marked by a blue or red triangle on each scaffold, depending on their orientation. Telomeric motifs are found in regions mapping to one end of chromosomes 1, 5 and 9 and to both ends of the remaining chromosomes. Scaffolds are named and listed based on their chromosomal assignments.



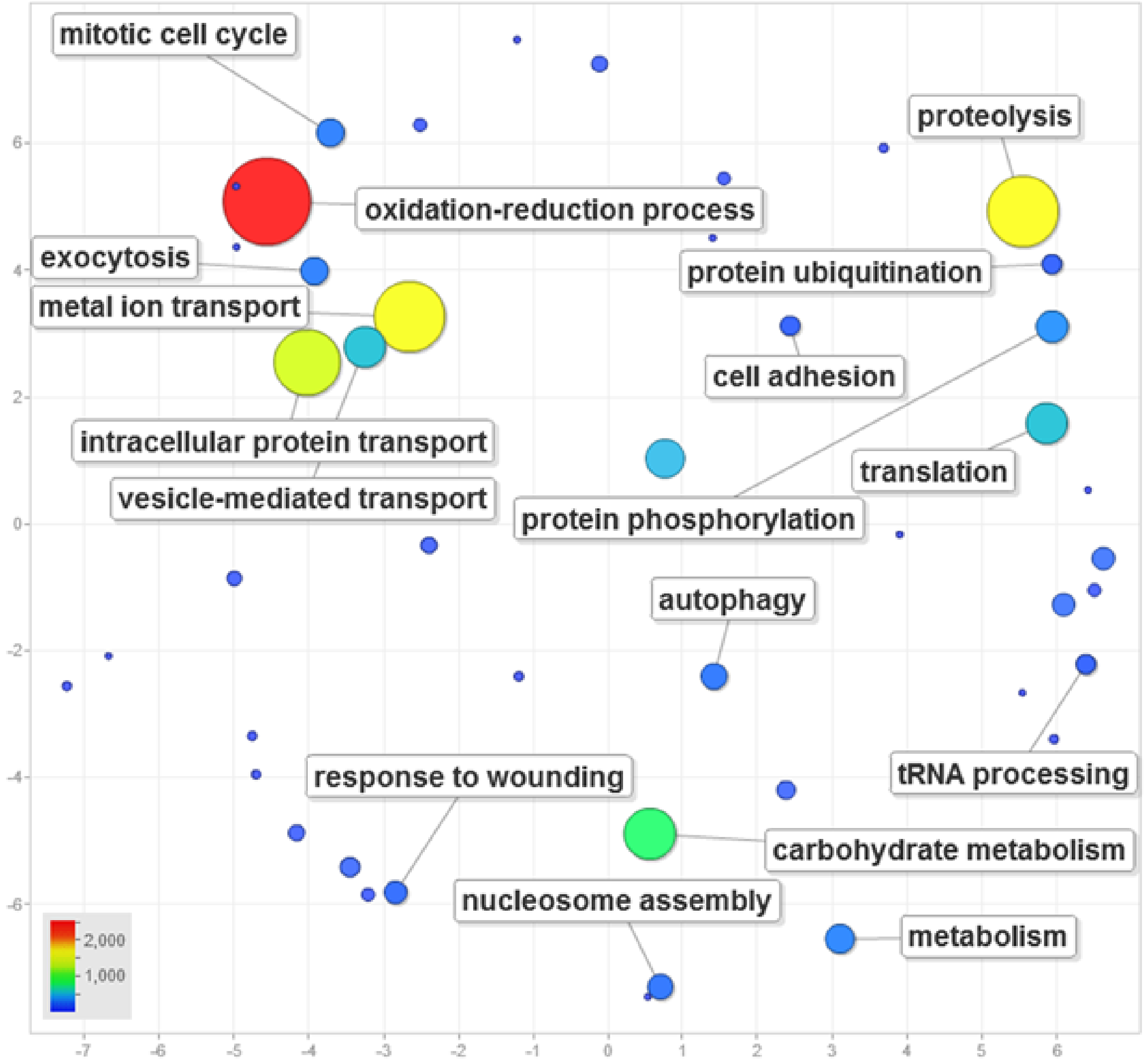
Supplementary Figure 9: Determination of length of repeat regions *vs.* count in the Tx430 hybrid assembly. Length of repeat regions were assessed by determining the length of masked regions after RepeatMasker screening.



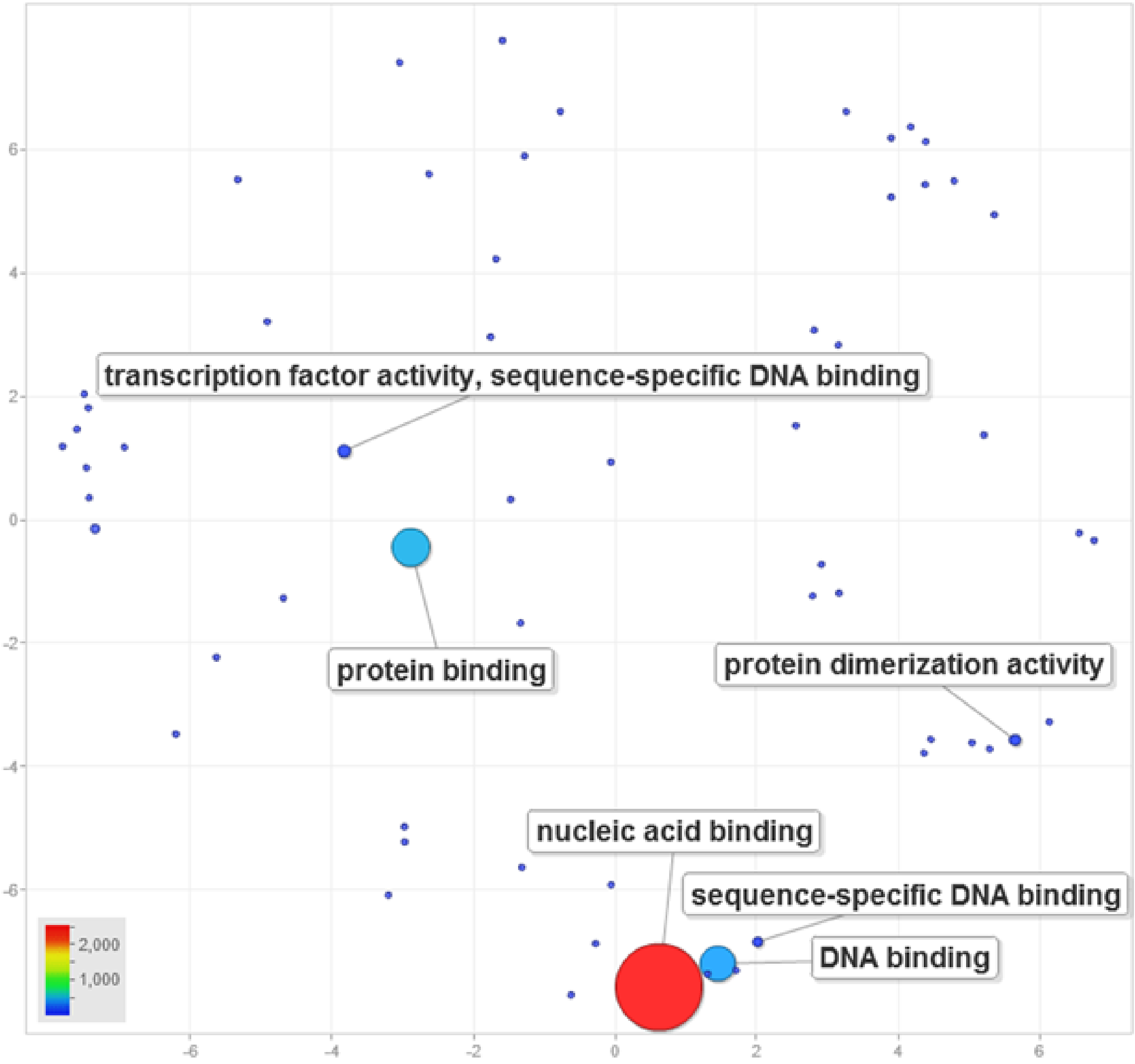
Supplementary Figure 10: Close-up view of one chromosome 6 inversion breakpoint region in BTx623. The *in-silico* map derived from the v3.0.1 BTx623 assembly is shown in green while the BTx623 DLS optical map is shown in blue. Overlapping individual Bionano molecules aligned to the DLS optical map and spanning the breakpoint area are shown below the map. The approximate location of the inversion breakpoint is marked by a red arrow.



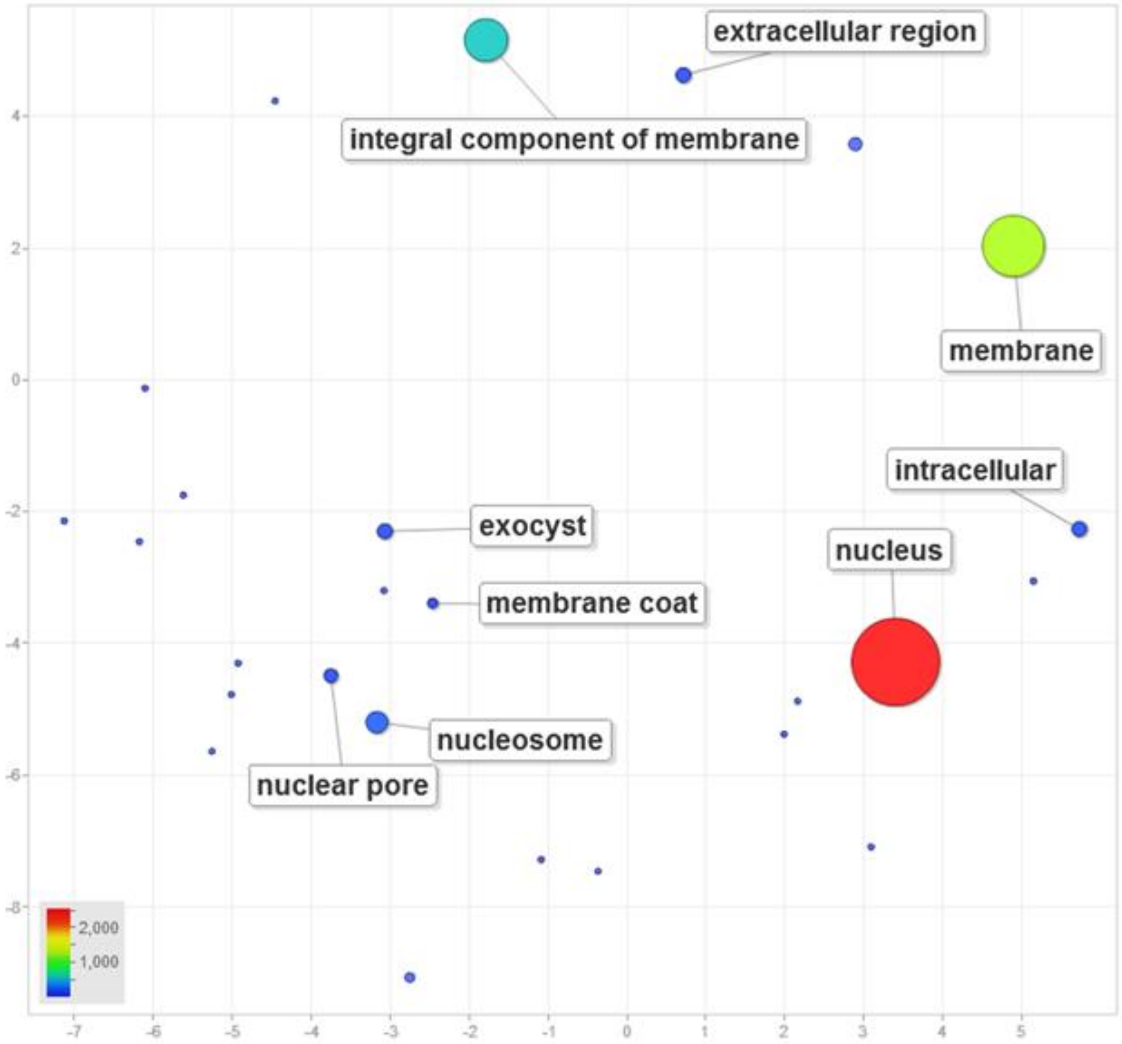
Supplementary Figure 11: BUSCO analysis for annotation quality assessment.



Supplementary Figure 12: GO-term enrichment for biological process in 718 Tx430 proteins that are not homologous to any BTx623 protein. Colors depict fraction of the proteins covered by a single GO term. Blue – low fraction, Red – higher fraction



Supplementary Figure 13: GO-term enrichment for molecular function in 718 TX430 proteins that are not homologous to any BTx623 protein. Colors depict fraction of the proteins covered by a single GO term. Blue – low fraction, Red – higher fraction



Supplementary Figure 14: GO-term enrichment for cellular component in 718 Tx430 proteins that are not homologous to any BTx623 protein. Colors depict fraction of the proteins covered by a single GO term. Blue – low fraction, Red – higher fraction

Supplementary Table 1: Summary of sequencing run metrics on the MinION (SR = "Short Reads"; LR = "Long Reads")

MinION Runs	Number of reads	Number of bases	Mean read length (bps)	N_{50} read length (bps)
SR1	622,099	4,644,995,691	7,467	8,542
SR2	727,563	5,652,265,725	7,769	8,786
SR3	123,706	980,098,593	7,923	9,613
SR4	416,303	3,635,049,741	8,732	10,170
SR5	578,142	4,536,332,937	7,846	8,968
SR6	1,136,111	7,336,281,268	6,457	7,228
SR7	1,059,720	6,740,546,727	6,361	7,124
LR1	63,842	562,551,609	8,812	19,934
LR2	24,401	231,228,623	9,476	21,447
LR3	43,534	1,000,162,973	22,974	27,974
LR4	4,440	114,776,296	25,851	38,037
LR5	22,355	523,144,528	23,402	36,180
LR6	41,228	862,021,880	20,909	34,713
LR7	89,690	2,158,820,365	24,070	33,336
LR8	135,416	2,950,632,392	21,879	32,525
LR9	95,855	1,755,347,777	18,313	28,246
LR10	157,482	3,167,308,207	20,112	30,954
LR11	233,125	4,695,207,834	20,140	29,869
LR12	152,273	1,562,164,979	10,259	15,639
LR13	184,229	2,354,548,651	12,781	19,272
LR14	303,600	4,101,399,523	13,509	20,198
LR15	66,484	1,432,778,163	21,551	32,928
LR16	96,917	2,308,041,410	23,815	38,180
LR17	110,361	1,905,096,315	17,262	28,734
LR18	44,016	820,398,292	18,639	32,656
LR19	48,872	1,084,571,470	22,192	35,118

Supplementary Table 2: Summary of assembly metrics ("LR" = Long reads only)

	CANU + SMARTdenovo (40X)	CANU + SMARTdenovo (60X)	CANU + SMARTdenovo (LR)
Number of Contigs	1,366	1,059	740
Total Length	606,505,989	611,320,497	628,023,803
Average Contig Length	444,001	577,262	848,681
Minimum Contig Length	8,781	7,314	16,729
Maximum Contig Length	14,256,537	15,767,440	18,368,286
N_{25} Contig Length	2,083,917	3,249,570	5,909,789
N_{50} Contig Length	852,709	1,186,175	1,920,445
N_{75} Contig Length	387,954	562,306	799,145

Supplementary Table 3: Summary of correction and assembly input metrics

	Number of reads	Number of bases	Mean read length (bps)	N_{50} read length (bps)
Total raw reads (>100bps)	6,527,158	66,488,480,580	10,186	12,585
Raw short reads (>2Kbps)	4,397,189	32,417,762,086	7,372	8,067
Raw long reads (>2Kbps)	1,762,807	33,637,416,971	19,082	27,335
Canu-corrected reads (>2Kbps)	5,115,181	47,898,319,758	9,364	11,088
Canu-corrected reads (>5Kbps)	3,555,340	42,331,080,779	11,906	12,928

Supplementary Table 4: Summary of the Tx430 DLS map generation and assembly

Input Molecules (filtered to >150Kbps):		Molecules Aligned to the Reference:	
Total Number of >150Kbps molecules	1,224,604	Total Number of Molecules Aligned	850,581
Total Length of >150Kbps molecules (Mbps)	340,107	Fraction of Molecules Aligned	0.695
N_{50} of >150Kbps molecules (Kbps)	286.205	Effective Coverage of Assembly (X)	263.428
Raw coverage of the reference (X)	464.531	Average Confidence	36.1
De novo Assembly:		SV Summary:	
Genome Map Number	79	Deletion	1,750
Total Map Length/Reference Length	0.982	Duplication-Inverted	120
Genome Map N_{50} (Mbps)	33.773	Insertion	2,327
Total Reference Length (Mbps)	732.152	Inversion Breakpoints	52
Total Number of Maps Aligned (Fraction)	32 (0.41)	Translocation - Interchromosomal	22
		Translocation - Intrachromosomal	6

Supplementary Table 5: Summary of the BTx623 DLS map generation and assembly

Input Molecules (filtered to >150Kbps):		Molecules Aligned to the Reference:	
Total Number of >150Kbps molecules	911,159	Total Number of Molecules Aligned	640,773
Total Length of >150Kbps molecules (Mbps)	256,923	Fraction of Molecules Aligned	0.703
N_{50} of >150Kbps molecules (Kbps)	293.343	Effective Coverage of Assembly (X)	211.172
Raw coverage of the reference (X)	350.916	Average Confidence	35.3
De novo Assembly:		SV Summary:	
Genome Map Number	44	Deletion	211
Total Map Length/Reference Length	0.988	Insertion	1,256
Genome Map N_{50} (Mbps)	34.617	Inversion Breakpoints	30
Total Reference Length (Mbps)	732.152	Translocation Breakpoints	17