

Chromosome-scale assembly of the yellow mealworm genome

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Supplementary Table 1: Genomic data

Nanopore reads from different runs on PromethION and MinION devices were merged and used together.

	BG (male pupa)	BG (male pupa)	BG (male pupa)	BO (male pupa)
Sequencing Technology	ONT Promethion	ONT Minion	HiSeq 4000	HiSeq 4000
Library Protocol	1D genomic DNA by ligation	1D gDNA by selecting for long reads	PCR free	Dovetail Hi-C
Nb of Reads	2,264,874	217,053	162,443,183 x 2	124,589,387 x 2
Read length (mean for ONT*)	19,129	9,763	151 x 2	151 x 2
Max Read length (only for ONT)	247,043	181,352		
Cummulative size	45,443,965,268		47,496,139,168	37,141,198,341
N50 (only for ONT)	34,818			
Coverage *	146 x		153x	119x
GC %	39.75%		39.29%	39.30%

* for a genome of 310Mb based on GenomeScope Profile

*ONT: Oxford Nanopore Technologies

Supplementary Table 2: Transcriptomic data

AA and AB samples are pools of 150 insects each and were already available for downloading on NCBI. The rest six samples were sequenced at Genoscope, Institut François Jacob in France.

	AA (pool of 150 insects) NCBI	AB (pool of 150 insects) NCBI	AE (female pupa)	AF (female adult)	AH (sterile larva)	AI (sterile male adult)	AJ (sterile juvenile)	AK (sterile male pupa)
Sequencing Technology	Illumina HiSeq 1000	Illumina HiSeq 1000	NovaSeq 6000	NovaSeq 6000	NovaSeq 6000	NovaSeq 6000	NovaSeq 6000	NovaSeq 6000
Library Protocol	Maxima H minus first strand	Maxima H minus first strand	TruSeq reversely-stranded	TruSeq reversely-stranded	TruSeq reversely-stranded	TruSeq reversely-stranded	TruSeq reversely-stranded	TruSeq reversely-stranded
Nb of Reads	48,301,407 x 2	65,990,251 x 2	77,007,578 x 2	60,203,841 x 2	71,885,055 x 2	62,293,152 x 2	64,087,701 x 2	78,125,398 x 2
Read length (x2 paired-end)	100 x 2	100 x 2	151 x 2	151 x 2	151 x 2	151 x 2	151 x 2	151 x 2
Cummulative size	9,389,557,947	12,814,810,793	22,909,528,124	17,922,385,942	22,909,528,124	18,447,453,373	18,884,945,164	22,815,151,154
Coverage *	30x	41x	73x	57x	73x	59x	60x	73x
GC %	34.69%	35.27%	45.28%	44.07%	44.74%	43.82%	44.26%	45.21%

* for a genome of 310Mb based on GenomeScope Profile

Supplementary Table 3: Metrics for long reads, contigs and scaffolds through different steps

	Long Reads	Long Reads (after YACRD)	Long Reads (after NECAT Pre- filtering)	NECAT Assembly	NECAT Assembly (after Polishing)	Haplotype (after Haplo- Merger2)	Polished Haplotype	Scaffolds (with Salsa2)	Final Assembly
# Contigs	2,481,927	2,372,861	250,277	527	527	187	187	138	¹¹¹ (109 nuclear + 2 mitochondrial)
Max Contig Length	247,043	200,175	155,166	8,326,708	8,396,068	20,298,552	20,297,691	47,681,210	47,681,210
Mean Contig Length	18,310	15,807	49,546	814,527	819,341	1,541,657	1,541,607	2,089,196	2,593,979
Cumulative size	45,443,965,268	37,508,352,859	12,400,190,549	429,255,720	431,792,740	288,289,869	288,280,515	288,309,015	287,931,689
N50 (L50)	34,818 (448,059)	31,788 (398,831)	49,311 (100,050)	2,426,793 (57)	2,452,408 (57)	6,812,004 (12)	6,811,711 (12)	22,440,466 (5)	22,440,466 (5)
N90 (L90)	11,951 (1,274,614)	10,097 (1,170,536)	37,464 (216,023)	559,678 (179)	562,731 (179)	1,377,763 (48)	1,377,747 (48)	5,674,206 (15)	5,674,206 (15)
auN	37,542	34,524	52,859	2,778,417	2,798,350	8,771,730	8,771,103	22,535,111	22,564,613
GC %	39.75%	39.43%	38.57%	36.73%	36.66%	36.71%	36.72%	36.72%	36.72%

Supplementary Table 4: Repeats

Repeats for *T. molitor* 2020 and *T. castaneum* were detected using same tools and parameters as for *T. molitor* 2021 (see main article). The portion of genome covered by repeats is 5-6% for the three assemblies.

	Tenebrio molitor 2021	Tenebrio molitor 2020	Tribolium Castaneum
assembly size	287,931,689 bp	280,780,514 bp	165,944,485 bp
contigs	111	31,390	2,082
GC level	36.72%	36.03%	33.86%
bases masked	17,298,313 bp (6.01 %)	14,784,139 bp (5.27 %)	10,284,212 bp (6.20 %)
	number of / length occupied / percentage of sequence elements*	number of / length occupied / percentage of sequence elements*	number of / length occupied / percentage of sequence elements*
DNA transposons:	29,182 / 5,584,839 bp / 1.94%	38,447 / 6,101,177 bp / 2.17%	21,026 / 5,757,743 bp / 3.47%
hAT-Charlie	354 / 75,994 bp / 0.03%	495 / 80,945 bp / 0.03%	129 / 20,631 bp / 0.01%
TcMar-Tigger	196 / 28,919 bp / 0.01%	293 / 37,247 bp / 0.01%	1,057 / 80,257 bp / 0.05%
SINEs:	539 / 32,334 bp / 0.01%	1,070 / 58,098 bp / 0.02%	281 / 31,050 bp / 0.02%
ALUs	1 / 50 bp / 0.00%	3 / 382 bp / 0.00%	1 / 45 bp / 0.00%
MIRs	2 / 65 bp / 0.00%	6 / 405 bp / 0.00%	0 / 0 bp / 0.00%
LINEs:	11,417 / 4,005,113 bp / 1.39%	12,754 / 3,472,876 bp / 1.24%	4,398 / 1,301,528 bp / 0.78%
LINE1	329 / 20,275 bp / 0.01%	634 / 37,990 bp / 0.01%	163 / 10,386 bp / 0.01%
LINE2	1,099 / 149,969 bp / 0.05%	1,381 / 168,228 bp / 0.06%	1,068 / 306,642 bp / 0.18%
L3/CR1	1,688 / 424,660 bp / 0.15%	2,181 / 455,200 bp / 0.16%	1,637 / 675,386 bp / 0.41%
LTR elements:	7,950 / 3,597,958 bp / 1.25%	10,290 / 2,306,092 bp / 0.82%	3,273 / 824,802 bp / 0.50%
ERV1	18 / 1,057 bp / 0.00%	43 / 2,179 bp / 0.00%	11 / 634 bp / 0.00%
ERV1-MaLRs	2 / 106 bp / 0.00%	4 / 224 bp / 0.00%	4 / 192 bp / 0.00%
ERV_classI	119 / 6,401 bp / 0.00%	279 / 14,905 bp / 0.01%	83 / 4,607 bp / 0.00%
ERV_classII	79 / 4,504 bp / 0.00%	150 / 8,170 bp / 0.00%	65 / 3,994 bp / 0.00%
Unclassified	1,790 / 228,410 bp / 0.08%	2,553 / 281,171 bp / 0.10%	547 / 76,839 bp / 0.05%
Small RNA	2,851 / 939,516 bp / 0.33%	612 / 75,855 bp / 0.03%	646 / 73,705 bp / 0.04%
Satellites	605 / 273,554 bp / 0.10%	404 / 78,560 bp / 0.03%	211 / 24,706 bp / 0.01%
Simple repeats	49,992 / 2,094,562 bp / 0.73%	47,642 / 1,874,361 bp / 0.67%	36,699 / 1,708,043 bp / 1.03%
Low complexity	11,960 / 575,359 bp / 0.20%	11,941 / 566,664 bp / 0.20%	10,271 / 504,269 bp / 0.30%

*most repeats fragmented by insertions or deletions have been counted as one element

Supplementary Method 1: Alignment filtering method using Arima Genomics pipeline (https://github.com/ArimaGenomics/mapping_pipeline)

Because Hi-C captures conformation via proximity-ligated fragments, paired-end reads are first mapped independently (as single-end reads) and subsequently paired in a later step. Hi-C reads and alignments contain experimental artifacts so the alignments need some additional processing. More precisely, we applied the script "filter_five_end.pl" to each bam file (Read1 and Read2) and afterwards paired the filtered single-end Hi-C reads using "two_read_bam_combiner.pl". Then, with Picard tools (<https://broadinstitute.github.io/picard/>) we added read groups to the combined BAM file (with the command AddOrReplaceReadGroups) and discarded any PCR duplicates present in the paired-end BAM file (with the command MarkDuplicates).

Finally, we converted bam files to bed format using the bamToBed command from the BEDtools package (<https://bedtools.readthedocs.io/en/latest/>) and set it as input to the SALSA2 pipeline. The restriction sites for the enzymes that were used for the Hi-C experiment were specified in the parameter -e (GATC,GCATGC in our case) of the script "run_pipeline.py".

Supplementary Method 2: Splitting Oases contigs

Assembly tools often erroneously merge sequences into one single contig and Oases is prone to this behaviour. To address this problem we used a homemade script that splits chimeric contigs. Splitting a contig into regions where different ORFs appear, or regions where abrupt shifts in read-coverage occur, could streamline the gene-prediction process. Based on combined resources such as the pileup-coverage, the research of ORFs (TransDecoder <https://github.com/TransDecoder/TransDecoder/releases>) and domains, this tool aims to split contigs in an adequate way so that sequences with different functional sites form different contigs.

Supplementary Method 3: Filtering Gmove predictions

Gmove predicted 24,870 genes with 27% of them being intronless.

While we are more confident in multi-exon gene predictions we become cautious with intronless genes as they can be transposable elements or false positive predictions caused by the fragmented alignment of transcripts to the assembly.

More particularly, we launched HMMER (version 3.2.1 with evalset set to 10e-5) for detecting known pfam domains and a DIAMOND analysis against nr database for protein hits (version 0.9.24 with --eval 10e-5, --unal 0).

Furthermore, RepeatModeler version 2.0.1 was used for screening *ab initio* repeats in the *T. molitor* assembly and 46.77% of the genome was masked. Then, we focused on overlaps between the predicted genes and repeats, using commands from BEDtools. Genes with exons highly covered by repeats (>90%) were automatically classified as repeats.

In parallel, we used "transposonPSI.pl" from "<http://transposonpsi.sourceforge.net/>" to align the virtual cDNA proteins of the 24,870 predictions against the TransposonPSI_08222010 library. We selected the single best transposonPSI match for each protein (from file proteins.fasta.TPSI.topHits) and tagged the corresponding genes as transposable elements. At this point, we excluded genes (single and multi exon) that were either highly covered by repeats (RepeatModeler) or TE tagged (TransposonPSI) without any blastp/pfam hit.

We also excluded intronless genes that were predicted only by RNA-Seq evidence (not any Coleoptera protein overlap) and at the same time composed of >80% untranslated regions (ratio UTR/(UTR+CDS)) without any pfam/blastp hit.

Additionally, we searched for overlaps between intronless predicted genes and CDS of proteic or mRNA evidence. Then, we discarded any intronless gene not accomplishing any of the following conditions:

1. A gene that is predicted from at least one mRNA and one protein evidence.
2. A gene that is predicted from mRNA transcripts of at least two different samples.
3. A gene that is predicted from at least a *T. molitor* protein (from Uniprot).

If none of the above criteria was met and a gene did not have any pfam/blastp hit either, then it was removed. After this filtering process we ended up with 21,435 gene predictions.

Supplementary Note 1: Additional RNA-seq samples used (NCBI accession name: PRJNA646689)

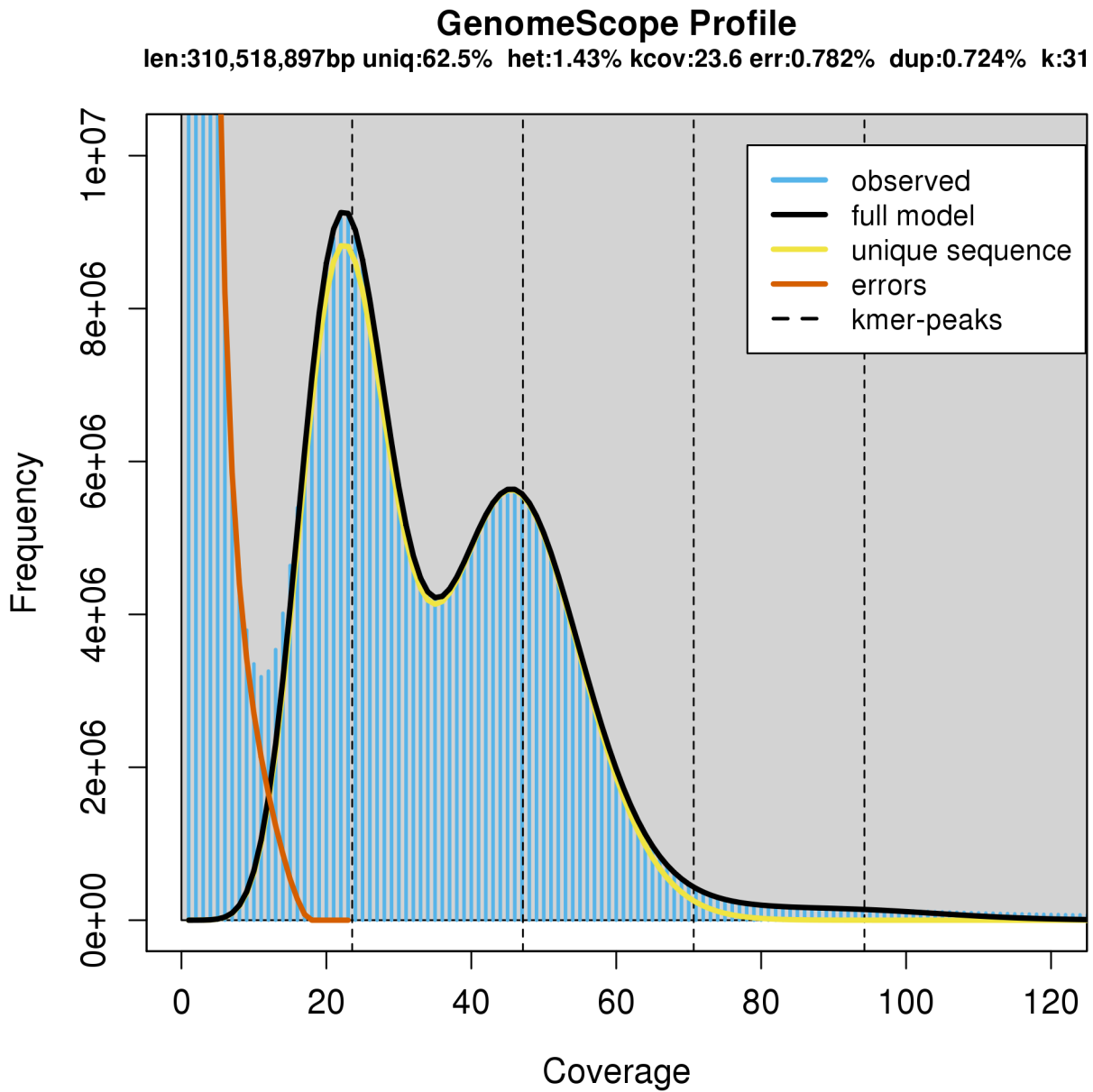
Design: RNA was extracted from 150 insects of various developmental stages (larvae, pupae, adults), sex (females and males) and physiological conditions. Physiological conditions were of four types: insects were challenged with either *Bacillus thuringiensis* (Pasteur Institute CIP53.1) or *Serratia Entomophila* (Pasteur Institute CIP102919), other insects were injected by phenobarbital (0,1%), control insects with no challenge and no injection. RNA was extracted by eurofins mwg operon.

Supplementary Note 2: Gmove

Gmove is an easy-to-use predictor with no need of a pre-calibration step. Briefly, putative exons and introns, extracted from alignments, are used to build a graph, where nodes and edges represent, respectively, exons and introns. Gmove extracts all paths from the graph and searches open reading frames that are consistent with the protein evidence.

Supplementary Figure 1: GenomeScope Profile for *T.molitor*

Genome size is estimated at ~310Mb with an heterozygosity rate of 1.43%.

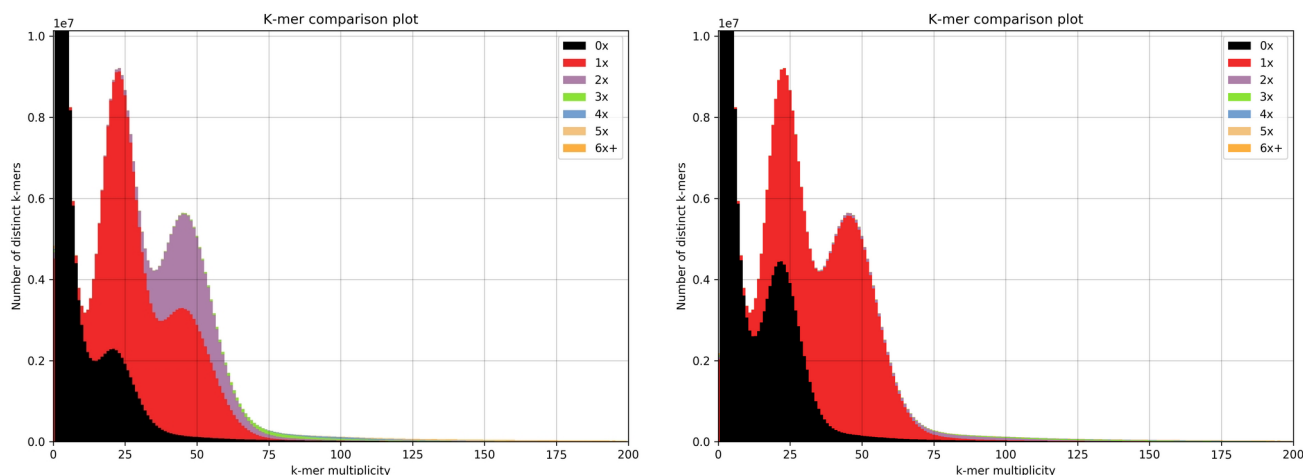


Supplementary Figure 2: K-mer plot before and after Haplomerger

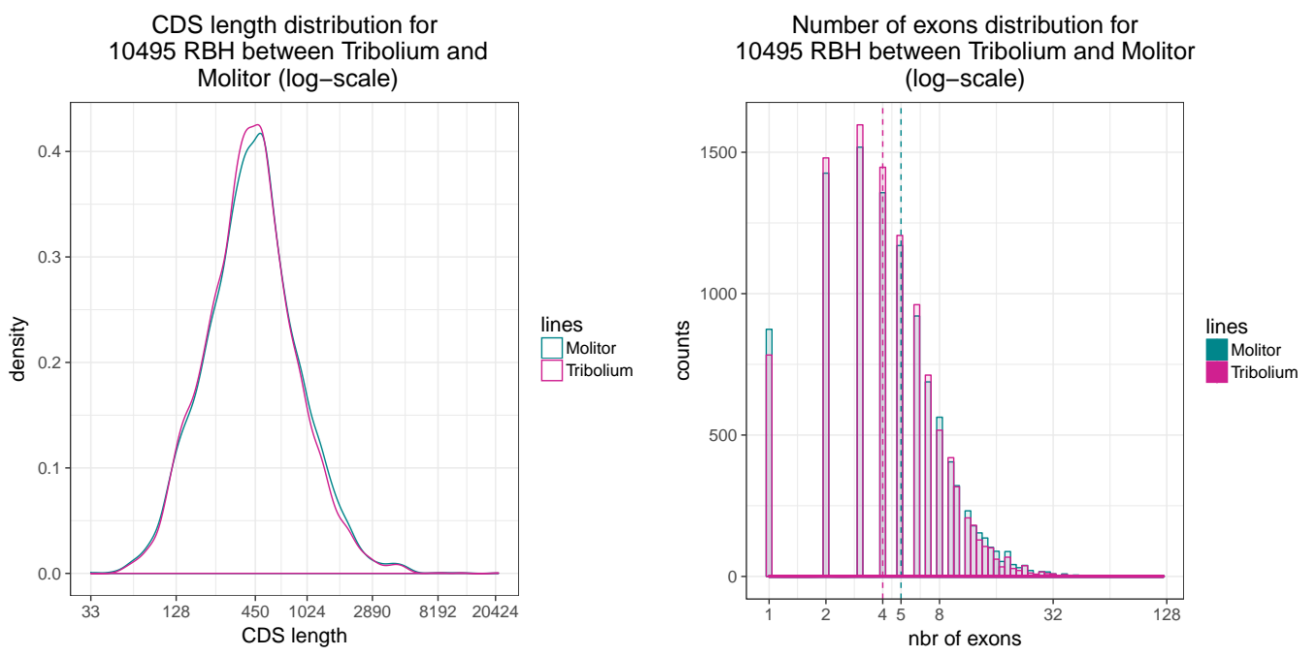
Haplomerger2 efficiently eliminated the duplicated part of the assembly (purple curve on image a)

a) Before Haplomerger

b) After Haplomerger



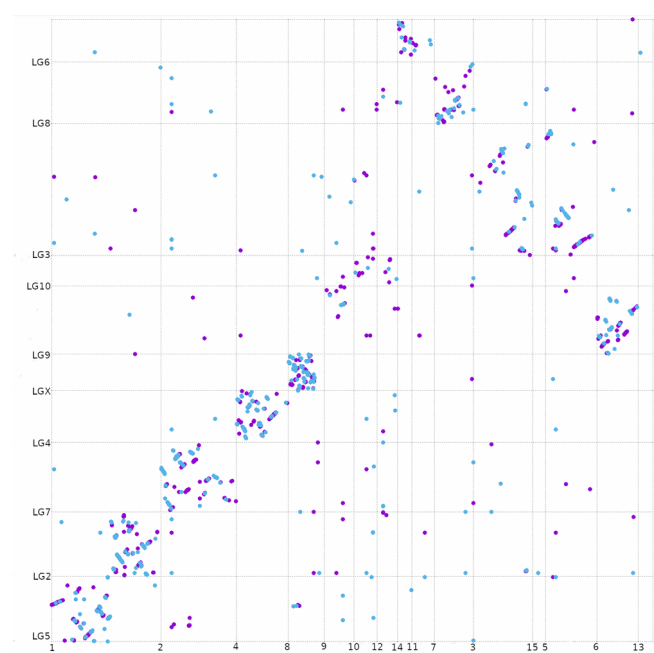
Supplementary Figure 3: Comparison of CDS lengths and number of exons of orthologous genes between T.molitor and T.castaneum



CDS lengths are log-transformed but we maintained real length values on the x-axis. The pink coloured curve corresponds to T.castaneum values and the turquoise to T.molitor. Mean CDS length is at 428 amino acids for T.molitor and 418 for T.castaneum. The trends of the two curves are quite similar.

Values for plotting are log-transformed but we maintained the real numbers on the axis. Pink and turquoise coloured curves correspond to T.castaneum and T.molitor, respectively. Dashed vertical lines represent the median values (5 exons for T.molitor and 4 for T.castaneum).

Supplementary Figure 4: Aligning T.molitor to T.castaneum



Alignment (using NUCmer) between the 15 longest scaffolds of T.molitor (x-axis) and the 10 chromosomes of T.castaneum (y-axis).

The alignment shows several clear associations between :

- scaffold_1 and LG5 together with LG2 (scaffold_1 as a fusion of 2 T.castaneum chromosomes),
- scaffold_2 and LG7
- scaffold_3, scaffold_5 and LG3 (as if chromosome LG3 underwent a fission in T.molitor)
- scaffold_4 and LG4
- scaffold_7 and LG8
- scaffold_6, scaffold_9 and LG9

as well as some more ambiguous associations between:

- scaffold_8 and LGX (and part of LG5)
- scaffold_10, scaffold_12 and LG10
- scaffold_11, scaffold_13, scaffold_14, scaffold_15 and LG6

Supplementary Figure 5: Position of the 142 bp satellite (TMSATE1) on scaffolds 15, 57, 98, 22 and their coverage by Illumina Reads

We performed a BLASTn analysis for the 142-bp satellite sequence (BLASTn overlap >80%, identity \geq 90%). The satellite was detected in 17 scaffolds (the 8 longest scaffolds 1-8 and 10, 12, 13, 15, 34, 45, 57, 98, 107) and extends to 174,807 bp accounting for 0.06% of the assembly. (According to RepeatMasker results, the satellite extends to 248,412 bp accounting for 0.08 % of the assembly).

While it ranges from 1 to 17 instances (average 4.35) in 14 scaffolds, it is highly present in scaffolds 15, 57, 98 (135, 534, 511 instances respectively).

More particularly, in scaffold 57, under the BLASTn constraints of overlap >80% and identity score \geq 90%, the satellite covers 77,501 bp (or ~47% of the scaffold's length) (Fig. 5e). According to RepeatMasker the satellite covers 84,915 bp or ~51.8% of the scaffold's length.

In scaffold 98 the region covered by the satellite measures 72,136 bp or almost 90% of the scaffold's length (Fig. 5g).

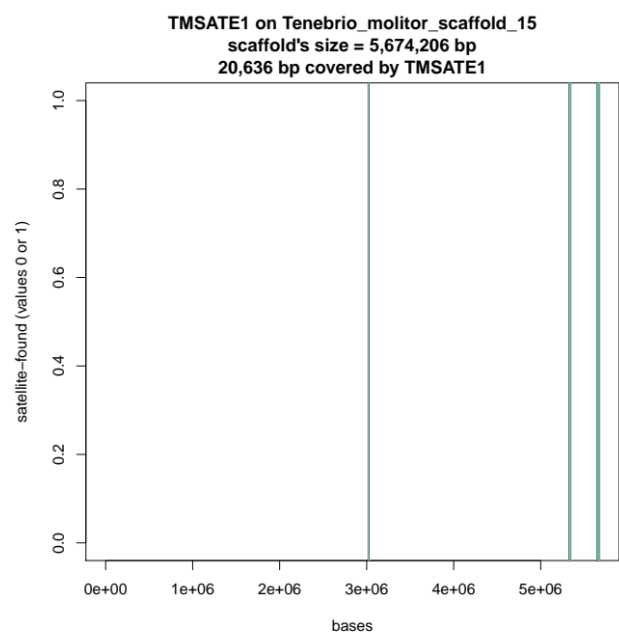
However, at the same time, scaffolds 57, 98 have an extremely high coverage by Illumina reads (Figures 5f, 5h),

probably indicating that different regions of the genome (containing the satellite) have been collapsed into single scaffolds (misassembled scaffolds due to erroneous sequence collapses).

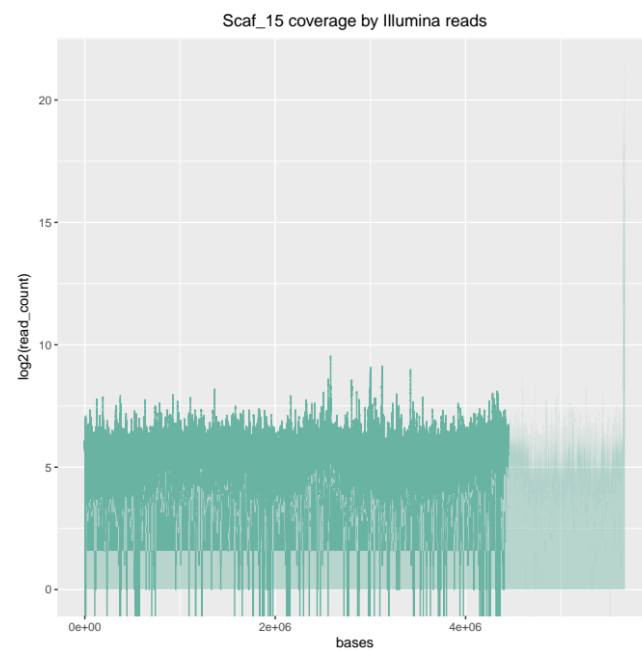
RepeatMasker detected the 142bp satellite (TMSATE1) also in scaffolds 9, 11, 14, 16, 17, 18, 20, 22. BLASTn didn't find it there simply because of the strict constraints of overlap and identity score we set.

It is worth noting that in scaffold 22 a variant sequence of the satellite accounts for 4% of scaffold's proper length (Fig. 5c). This portion is much higher than the median 0.02%, calculated on scaffolds that have the satellite (excluding the extreme cases of scaffolds 57, 98).

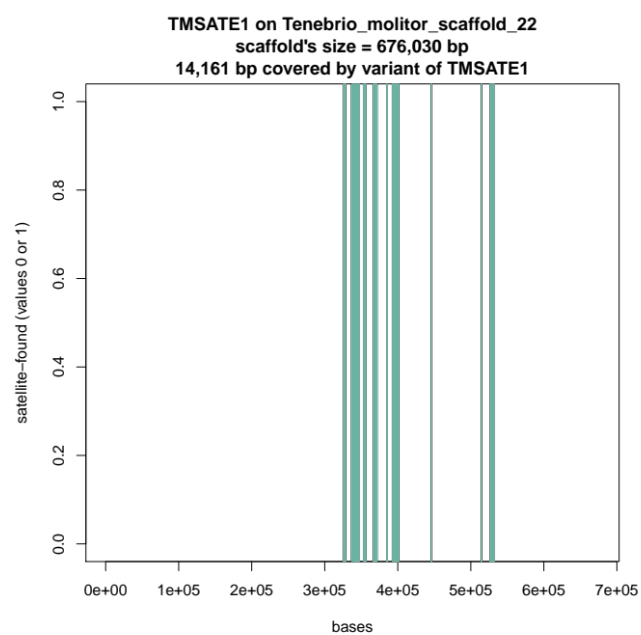
a



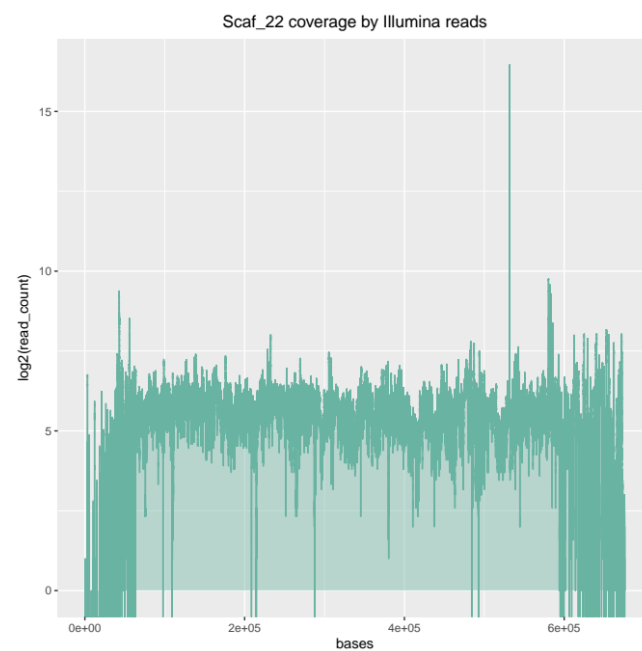
b



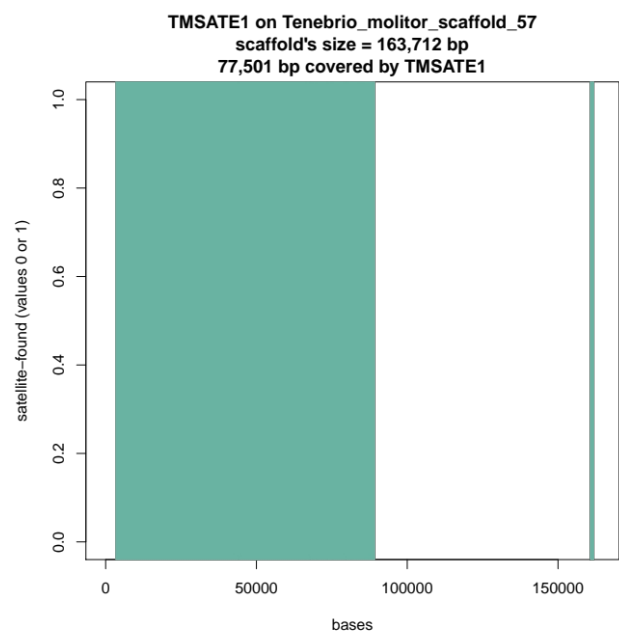
c



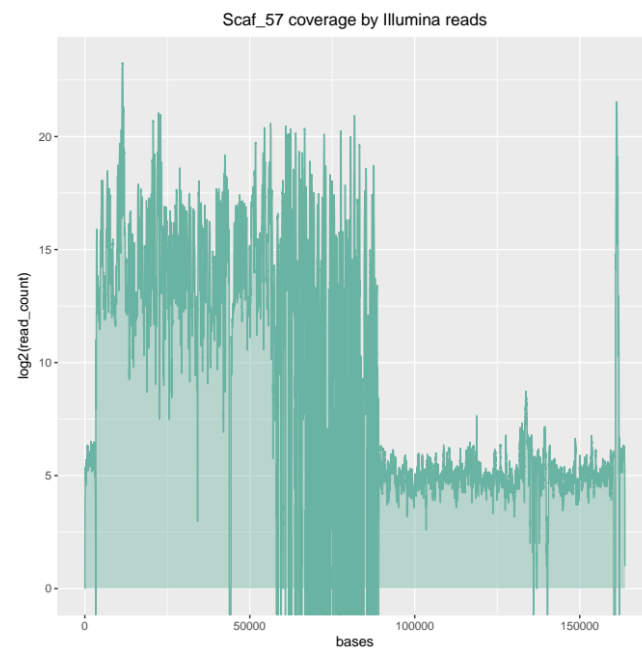
d



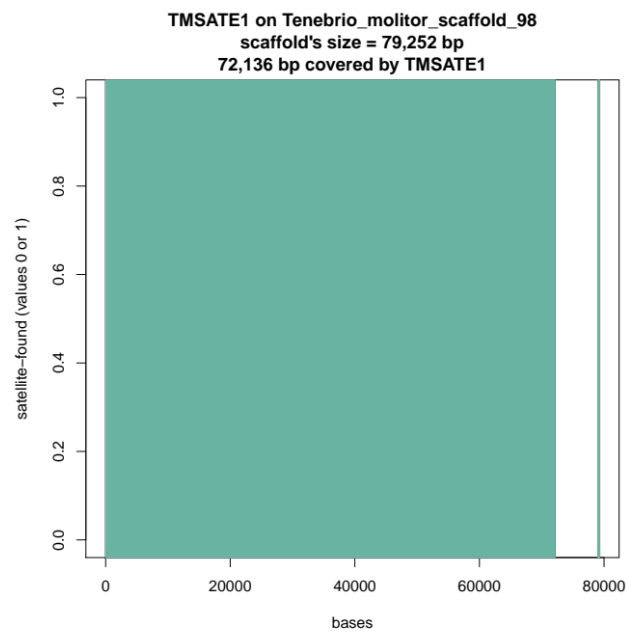
e



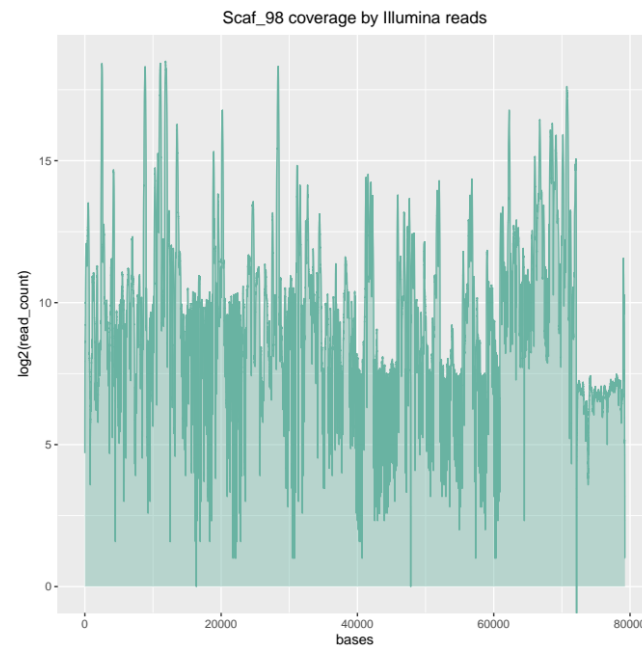
f



g

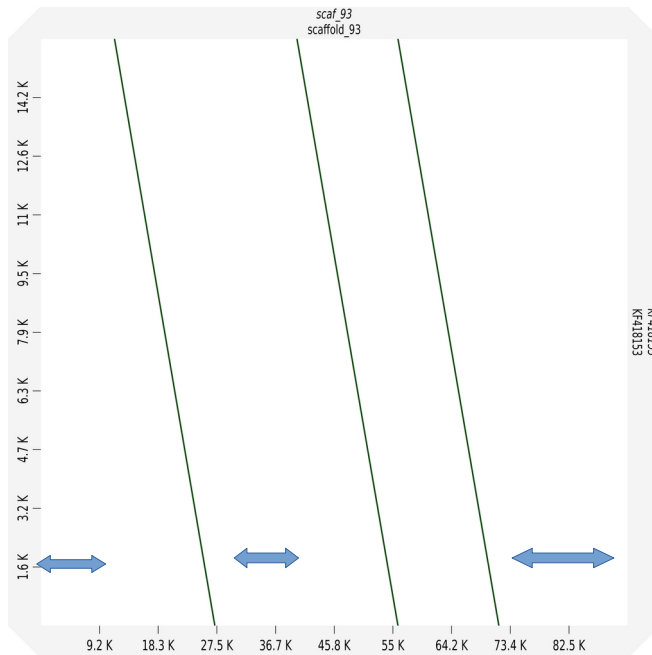


h



Supplementary Figure 6: Presence of mitochondrial genome on scaffold 93

We aligned, with minimap2, the mitochondrial genome of *T. molitor* (with name KF418154 at NCBI) to the assembly. Then, we plotted the alignment using the d-genies platform of Toulouse (<http://dgenies.toulouse.inra.fr/>) (Fig. 6a).



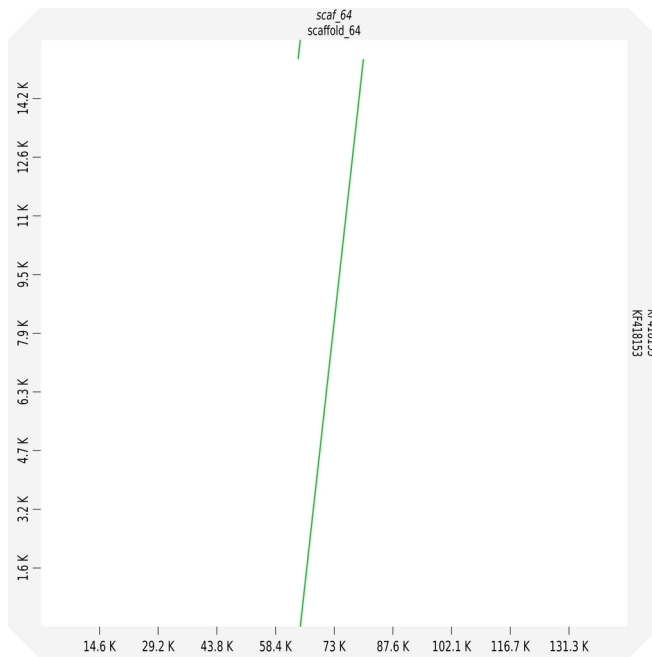
The figure on the left shows the alignment between the scaffold 93 (x-axis) and the mitochondrial genome (y-axis). The two sequences align with identity score 85-89% at three regions of the scaffold 93 (represented by green lines).

Then, we extracted the regions of scaffold 93 where mitochondrial genome did not align (represented by blue double arrows) and aligned the latter separately to each of these regions. The alignment identity scores were lower this time (lines in red text at the Table 4, right below).

Supplementary Table 5: Details for the alignment between the mitochondrial genome and the scaffold 93

Mito seq	Length of mito seq (bp)	Start position on mito seq	End position on mito seq	Strand	Target	Length of target seq (bp)	Start position on scaffold	End position on scaffold	Length of matched seq (bp)	Length of aligned seq (bp)	Identity Score
KF418153	15785	1	12188	-	Scaf_93: 1-11513	11513	21	11511	8938	12209	73%
KF418153	15785	16	15784	-	Scaf_93	91698	11513	27172	13564	15773	85%
KF418153	15785	1	15784	-	Scaf_93: 27172-40025	12854	17	12852	11063	15785	70%
KF418153	15785	1	15784	-	Scaf_93	91698	40025	55803	14095	15793	89%
KF418153	15785	1	15784	-	Scaf_93	91698	55805	71572	13954	15788	88%
KF418153	15785	1	15784	-	Scaf_93: 71572-91698	20127	3	13020	10779	15785	53%

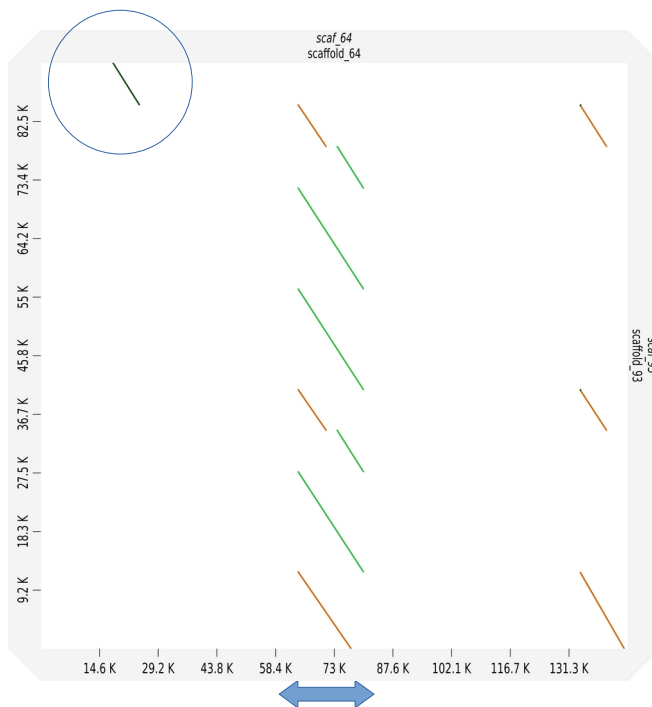
Supplementary Figure 7: Presence of mitochondrial genome on scaff 64



The figure on the left shows the alignment between the scaffold 64 (x-axis) and the mitochondrial genome (y-axis). The two sequences align with identity score 50-75%, lower than this to scaffold 93.

Supplementary Figure 8: Alignment of scaffolds 93, 64

The figure below shows how scaffolds 93 and 64 align to each other.



Scaffold 64 and 93 lie on x-axis and y-axis, respectively.

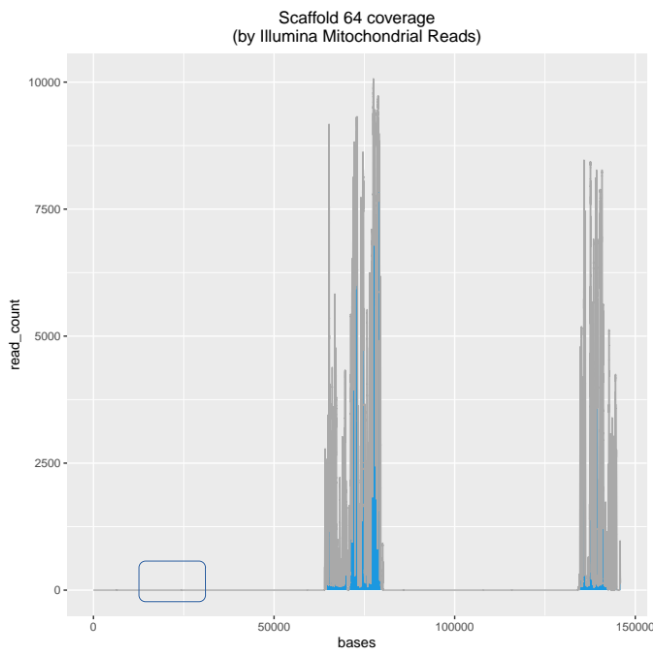
The part in the middle (blue double arrow) corresponds to the 15kb mitochondrial sequence while we also observe a better alignment between the two scaffolds located somewhere at the beginning of the scaffold 64 (dark green line in circle) and at the end of scaffold 93. The two sequences align also at the end of scaffold 64.

From the last line of the Table 4 (in red text) we observe that the mitochondrial sequence aligned from position 71,575 to 84,595 on scaffold 98. This means that there are almost 7kb bases at the end of scaffold 98 where mitochondrial genome does not align to and this region seems to be the common part between scaffolds 64 and 98 that we see on the figure in dark green line.

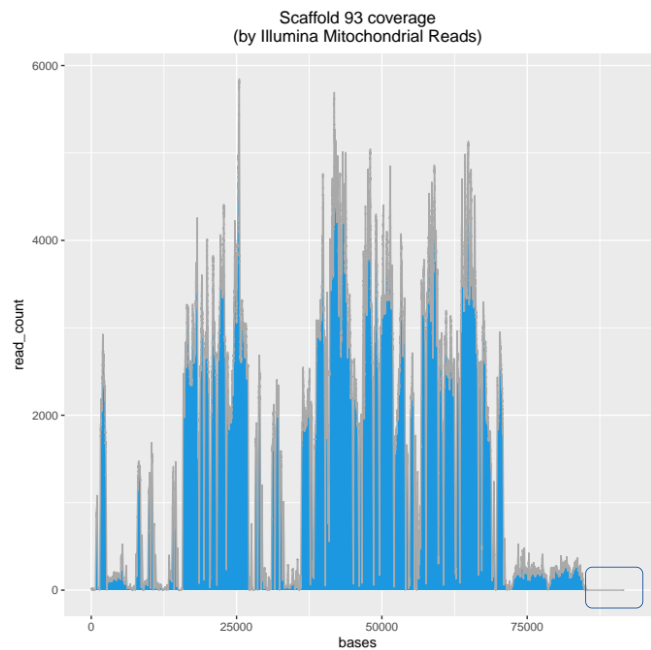
Supplementary Figure 9: Coverage of scaffolds 64, 93 by Illumina mitochondrial reads

We also aligned ,with BWAmem (parameter -B 8, identity score 90% and 80% overlap), Illumina reads to the mitochondrial sequence KF418153 in order to create a “pool” of high confidence mitochondrial reads. The figures below show the coverage of scaffolds 64 and 93 by mitochondrial reads. We confirm, once again, the existence of a common sequence between scaffolds 64 and 98 (represented by a rectangular on the figures below) that is 7kb long and where mitochondrial reads do not align to. This sequence could be an unknown ,so far, part of *T. molitor*’s mitochondrial genome.

The 7kb region is located at the beginning of the scaffold 64



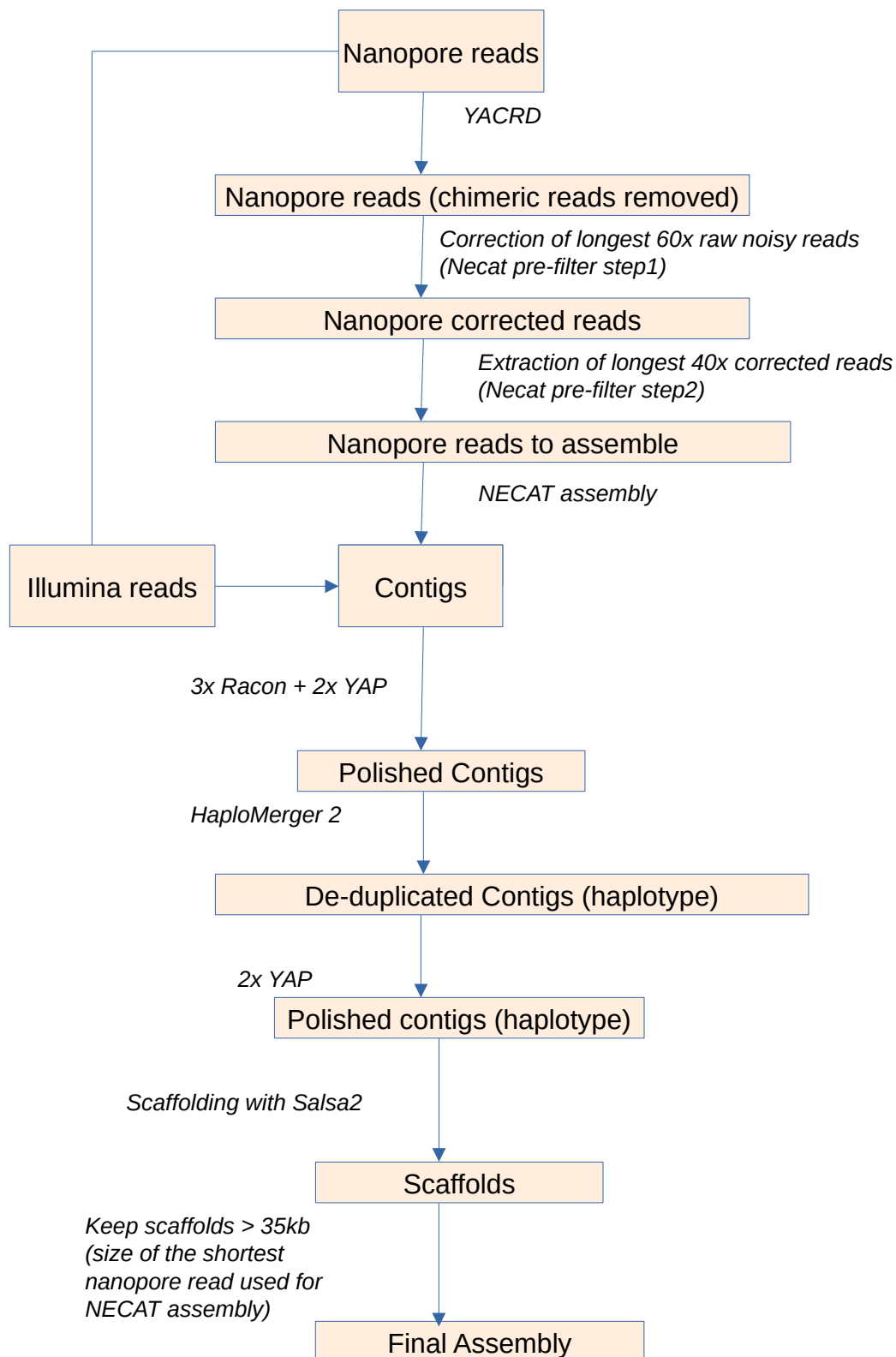
The 7kb region is located at the end of the scaffold 93



Supplementary Table 6: Samples' accession numbers

Sample Type	Sex and Developmental stage	Sample Name	Sequencing Technology	Study Accession	Submission Accession	Sample Accession	Experiment Accession	Run or Analysis Accession
DNA	Male pupa	CPD_BG_ONT_1_FAL24795_A	Oxford Nanopore	ERP128758	ERA4143595	ERS6344234	ERX5504889	ERR5859001
DNA	Male pupa	CPD_BG_ONT_1_PAD99440_A	Oxford Nanopore	ERP128758	ERA4143595	ERS6344234	ERX5504890	ERR5859002
DNA	Male pupa	CPD_BGOSDE_6_HFHC5BBXY.12B	Illumina PCR-free	ERP128758	ERA4143595	ERS6344234	ERX5504891	ERR5859003
DNA	Male pupa	CPD_BGOSDE_4_HFWM7BBXY.12B	Illumina PCR-free	ERP128758	ERA4143595	ERS6344234	ERX5504892	ERR5859004
DNA	Male pupa	CPD_BOOSDF_8_HGJYYB	Hi-C		ERA4185802		ERX5513830	ERR5870148
RNA	Female pupa	CPF_AEOSRB_1_CVTP3.12BA090	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508089	ERR5862256
RNA	Female pupa	CPF_AEOSRB_4_H2TK3DSXY.12B	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508095	ERR5862262
RNA	Female adult	CPF_AFOSRB_1_CVTP3.12BA091	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508090	ERR5862257
RNA	Female adult	CPF_AFOSRB_4_H2TK3DSXY.12B	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508096	ERR5862263
RNA	Sterile larva	CPF_AHOSRB_1_CVTP3.12BA093	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508091	ERR5862258
RNA	Sterile larva	CPF_AHOSRB_4_H2TK3DSXY.12B	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508097	ERR5862264
RNA	Sterile male adult	CPF_AIOSRB_1_CVTP3.12BA094	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508092	ERR5862259
RNA	Sterile male adult	CPF_AIOSRB_4_H2TK3DSXY.12B	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508098	ERR5862265
RNA	Sterile juvenile	CPF_AJOSRB_1_CVTP3.12BA095	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508093	ERR5862260
RNA	Sterile juvenile	CPF_AJOSRB_4_H2TK3DSXY.12B	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508099	ERR5862266
RNA	Sterile male pupa	CPF_AKOSRB_1_CVTP3.12BA096	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508094	ERR5862261
RNA	Sterile male pupa	CPF_AKOSRB_4_H2TK3DSXY.12B	NovaSeq	ERP128775	ERA4146275	ERS6348205	ERX5508100	ERR5862267
-	Genome Assembly + Annotation	-	-	ERP128837	-	ERS6376675	-	ERZ2140416

Supplementary Figure 10: Assembly workflow



Supplementary Figure 11: Annotation workflow

