The yellow mealworm (*Tenebrio molitor*) genome: a resource for the emerging insects as food and feed industry

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Abstract

The yellow mealworm, the larval stage of the darkling beetle *Tenebrio molitor*, shows great promise as an alternative source of animal protein. Herein we present the 312 Mb draft genome assembled using 10x Genomics linked-read technology to inform research efforts and to provide resources to optimise yellow mealworm for mass production and consumption. The genome with a contig N50 of 39,478 bp contains 89% of conserved arthropod genes among the >20,000 genes assembled (complete and partial genes). This draft assembly represents a valuable resource to understanding *T. molitor* biology as a means of producing alternative, sustainable protein for the growing population and in the face of changing climates.

Keywords: darkling beetle, genomics, sequencing, assembly, linked-reads

1. Introduction

Global food demands are rising at an alarming rate, and the lack of reliable, sustainable protein sources are an emerging food security issue across the world (FAO, 2017). Thus, alternatives to traditional protein production are a necessity. One such alternative is the use of mass-produced insects for food and animal feed. While great strides are being made in mass rearing insects for consumption, many challenges associated with commercial production remain, including the optimisation of desirable life history traits such as growth rate and fecundity. Historically a time-consuming endeavour through trial-and-error selective breeding, trait optimisation can now be significantly facilitated by studying and manipulating an organism's most basic feature: its genome.

Beetles (Coleoptera) make up nearly a quarter of Earth's biodiversity and possess complex evolutionary histories, as well as a remarkable richness in morphology and lifestyle (Barton *et al.*, 2011; Beutel and Leschen, 2014; McKenna *et al.*, 2015; Zhang *et al.*, 2011). Relatively few beetle genomes have been made public (McKenna, 2018), with the first published genome belonging to the stored grain pest, the red flour beetle *Tribolium castaneum* (Coleoptera:

Tenebrionidae) (McKenna, 2018; Richards *et al.*, 2008). Similarly, the darkling beetle *Tenebrio molitor* (Coleoptera: Tenebrionidae) and its associated larval form, the yellow mealworm, is a relative of *T. castaneum* and also considered a stored grain pest. However, the yellow mealworm possesses many qualities that can benefit a wide range of agricultural applications. High protein content and relative ease in rearing have made yellow mealworms a popular staple for the exotic pet food market for over 60 years. More recently, these larvae have been shown to be a suitable dietary supplement in the aquaculture and poultry industries (Choi *et al.*, 2018; Henry *et al.*, 2018; Veldkamp and Bosch, 2015). In addition, mealworm colonies are capable of breaking down organic materials, including mycotoxins (Van Broekhoven *et al.*, 2017), and excreting large quantities of nitrogen-rich frass, making them ideal producers of organic fertiliser (Poveda *et al.*, 2019). Lastly, there is evidence that farming mealworms is more sustainable than traditional livestock (Oonincx and De Boer, 2012).

T. molitor belongs to the family of detritivorous beetles Tenebrionidae, the seventh-most speciose taxon in Coleoptera comprising about 20,000 species in 2,300 genera worldwide (Zhang *et al.*, 2011). Although it remains unclear how distantly related they are, some estimates have placed

the respective most-recent-common ancestors of the tribes Tenebrionii and Tribolini circa 120 and 100 million years ago (Kergoat *et al.*, 2014), suggesting at least 100 million years of divergence. Due to this long divergence, existing genomic resources among Coleopterans are insufficient to inform breeding efforts in *T. molitor*.

Here, we present a draft genome assembly for the darkling beetle *T. molitor*. Our work adds to a growing body of genetic resources for *T. molitor*, which currently include a mitochondrial genome (Liu and Wang, 2014), transcriptomes (Liu *et al.*, 2015; Zhu *et al.*, 2013), gut microbiome (Brandon *et al.*, 2018; Jung *et al.*, 2014), expression pattern analyses (Johnston *et al.*, 2014; Oppert *et al.*, 2012), and gene and peptide annotations (Liu *et al.*, 2015; Prabhakar *et al.*, 2007). Recently it has been demonstrated that yellow mealworms can be artificially selected for commercially desirable traits (Morales-Ramos *et al.*, 2019). This finding highlights the need for a genome to contextualise and interpret selection outcomes, as well as to explore more efficient means of trait optimisation. Genomic sequencing and bioinformatic-based genome assembly terms mentioned in this article are explained in Supplementary material S1.

2. Methods

Unless specified otherwise, default settings were used for all analyses. Figures were made with RStudio (RStudio Team, 2015) using the library *ggplot2* (Wickham, 2016). Raw data and final draft assembly are available in NCBI SRA database (BioProject PRJNA579236).

Sequencing

Genomic DNA (gDNA) was extracted from the head of a single adult male *T. molitor* Stoneville strain (Morales-Ramos *et al.*, 2019) using the Qiagen DNeasy Blood and Tissue Extraction kit (Qiagen, Germantown, MD, USA). Library preparation, sequencing, and read demultiplexing were carried out at the Indiana University (IU) School of Medicine Center for Medical Genomics Core Facility (Indianapolis, IN, USA). Quality and quantity of gDNA was first evaluated by TapeStation and Qubit with a recommendation of a molecular size range of >50 kilobases (kb). Then, 10 ng of gDNA is prepared following the Chromium Genome Reagent Kit v2 User Guide, CG00043_RevB (10x Genomics, Inc., Pleasanton, CA, USA).

Along with the gel beads in emulsion (GEMs) and partitioning oil, the master mixture containing gDNA is dispensed onto a Genome Chip in separate wells, and the chip loaded to the Chromium Controller for GEM barcoding, followed by post GEM clean-up, and library preparation. The Chromium Controller prepared sequencing libraries by employing 10x Gemcode™ technology. In short, GEMs captured and tagged high-molecular-weight gDNA

molecules with unique barcodes. Short reads originated from a given GEM can be computationally linked by the shared barcode to provide resolution at larger genomic scales, thereby improving assembly accuracy. In addition, since the probability of both copies at a given diploid locus receiving the same barcode is very small, the Gemcode™ technology also enables the parsing of allelic information.

The resulting library was sequenced using a custom program for 2×150 bp paired-end sequencing on Illumina NovaSeq 6000 (200 cycles, Illumina, San Diego, CA, USA). Paired-end technology allows a given genomic region to be sequenced from both complementary strands to improve assembly accuracy.

De novo assembly

Paired-end raw reads were demultiplexed with the longranger mkfastq pipeline (10x Genomics) and assembled using Supernova v2.0.0 (Weisenfeld *et al.*, 2017) on the IU Carbonate supercomputing cluster housed at Indiana University Bloomington Data Center (Bloomington, IN, USA). The effects of coverage (assuming a genome size of 508 Mb for *T. molitor* estimated from gametic nuclear DNA content; see Juan and Petitpierre 1991 and Gregory *et al.*, 2007) on assembly quality were assessed by instructing Supernova to use different number of reads through the maxread parameter (Table 1). Per recommendations by 10x Genomics support, scaffolds ≥1000 base pairs (bp) were retained in all assemblies. Option ‘pseudohap2’ was selected to output assembled haplotypes, representing haploid copies of the genome, separately. We used the program jellyfish (Marcais and Kingsford, 2011) to generate frequency distributions of unique k-mers in the unpaired raw reads after quality trimming per recommendations by 10x Genomics. Then we used GenomeScope (Vurture *et al.*, 2017) to estimate from the k-mer distributions the genome size and repeat content.

Table 1. Values of the maxread parameter used by the Supernova assembler. The assembler uses a variety of reads that are calculated or approximated to be some coverage amount of the genome, and calculates that percentage as a function of the total number of reads generated.

Coverage	Maxread	% reads used
50	84,666,667	25.9
56	94,826,667	29
60	101,600,000	31
75	126,669,732	38.7
98	166,666,667	50.9
118	200,005,898	61.1
130	220,008,464	67.2
193	327,244,832	100

Comparing assemblies

Each assembly using a varying number of reads was evaluated for quality based on scaffold N50, contig N50, and number of scaffolds ≥ 1 kb as well as compressed scaffolds that are ≥ 10 kb. Scaffold compression replaced continuous stretches of ambiguous bases, abbreviated Ns, by a single N. Ambiguity is a result of low read coverage or missing data. Assembled genome size was estimated from the distribution of k-mers in reads, raw coverage was calculated by dividing the number of bases in reads prior to barcode trimming by the estimated genome size, and effective coverage was calculated by averaging base graph edges of length 1000–2,000 bp k-mers that appear to be diploid, and GC content was assessed. Maximum scaffold length, the number of ambiguous bases (Ns) per 100 kb, scaffold N50, N75, L50 and L75 of uncompressed assemblies were obtained from the program Quality Assessment Tool (QUAST) v5.0.0 (Mikheenko *et al.*, 2018). We then examined the relationship between read number and metrics used to assess genome assembly quality with regression models in RStudio, and selected the assembly with high contiguity and low ambiguity (small number of Ns) as our assembled genome for further analyses.

Assembly completeness

For the focal assembly, gene content completeness was estimated by the program Benchmarking Universal Single-Copy Orthologs (BUSCO) v3.0.2 (Simao *et al.*, 2015; Waterhouse *et al.*, 2018) using curated ortholog databases of Eukaryota, Arthropoda, Insecta and the insect superorder Endopterygota. Completeness is a measure of assembly quality based on the proportions of expected conserved protein-coding genes recovered from the assembly.

Repetitive DNA

RepeatMasker v4.0.7 (Smit *et al.*, 2013) was used to screen for repetitive elements, using a *Tenebrio*-specific database. We also performed local BLAST searches for the 142-bp satellite motif repeats of which reportedly make up about half of the *T. molitor* genome (Petitpierre *et al.*, 1988) using the CLC Genomics Workbench v. 11.0.1 (Qiagen) in which the length fraction of the read to map was 0.5 and similarity fraction of 0.8.

Gene model prediction

Predictions of assembled sequences were done using AUGUSTUS v3.3 (Stanke and Waack, 2003) with the *T. castaneum* v.2012 gene database as a reference. Two analyses were performed predicting complete genes (parameter *genemodel* = complete) and both partial and complete genes simultaneously (*genemodel* = partial). AUGUSTUS modelled probability distributions for different

sequence types (e.g. coding DNA, mRNA, and amino acid) and gene components (e.g. exons and introns), and applied the model to the assembly to generate predictions. Partial predictions were missing certain components based on training data (e.g. partial genes = missing one or more exons), therefore incomplete.

Mitochondrial genome

The mitochondrial genome (mtDNA) was isolated from the assembly using a local BLAST search in CLC Genomics Workbench (Qiagen) with the published *T. molitor* mitochondrial genome (accession number KF418153.1) (Liu and Wang, 2014). A consensus mtDNA sequence was extracted and annotated for the 13 mitochondrial-specific genes (Liu and Wang, 2014) to confirm its presence in our assembled genome.

3. Results

Raw reads

A total of 327,244,832 reads were generated with a read quality distribution (Figure 1) wherein 90.6% of all reads had an average Q score between 29–41, which correspond to a base call accuracy between 99.9–99.99%. Q scores, also known as Phred quality scores, reflect the probability that a given DNA base in a read is incorrectly determined by a sequencer. Most reads (96.99%) did not contain ambiguities (Ns). Estimates of haploid genome size from 21-, 30-, 48-, and 74-mers ranged 221–272 Mb (Table 2).

Figure 1. Sequencing quality of all raw reads expressed in Q scores. Over 90% of reads scored 29 or higher on average, indicating the majority of reads are of good quality with high base-calling accuracy (99.9–99.99%).

Table 2. GenomeScope's estimates of haploid genome size and repeat content from raw reads based on k-mer size. Estimates were given as a range (min-max). Full genome sizes were calculated by doubling haploid estimates.

k	Haploid genome size (Mb)	Full genome size (Mb)	Repeat content size (Mb)	Repeat content (%)	Model fit
21	244.5-245	489-490	76.7-76.8	31.4-31.3	92.07-95.03%
30	221-221.9	442-443.8	40.5-40.7	18.3-18.3	94.69-96.86%
48	271.7-271.9	543.4-543.8	77.1-77.1	28.4-28.4	96.85-99.06%
74	261.4-262.2	522.8-524.4	58.7-58.9	22.5-22.5	97.40-99.67%

Comparing assemblies

We assessed the impact of the number of reads used for the assembly on the general outcome of the assembly. Most metrics of contiguity increased logarithmically with read number, including compressed assembly size, the number of scaffolds ≥ 10 kb, maximum scaffold size, and scaffold N50 and N75 (Table 3; Figure 2A-D). On the other hand, the number of scaffolds of all sizes and scaffold L50 and L75 decreased exponentially with increasing numbers of reads (Table 3; Figure 2B, D). Uncompressed assembly size and number of Ns per 100 kb scaled linearly with read number, while estimated genome size increased exponentially (Table 3; Figure 2A, F).

Based on these results, we selected the assembly containing approximately 130x coverage that scored well in contiguity metrics, with relatively high N50 and N75, low L50 and L75, the largest scaffolds, the greatest number of scaffolds ≥ 10 kb, and low total scaffold count with relatively few Ns (Figure 2).

The 130x assembly

Statistics for the 130x assembly are provided in Table 4. Using CLC Genomics Workbench, we mapped all raw reads (i.e. includes reads not used in the original assembly) back to this assembly to approximate how many reads were actually used in the assembly. Overall, 306,790,136 reads, or 93.75% of total raw reads were mapped successfully (average mapping coverage across scaffolds = 238.2). The assembled genome contains 36.04% GC content, within the range of known insect genomes (Archak *et al.*, 2007). The uncompressed assembly contained 0.32% Ns estimated from the count of Ns per 100 kb. There were 20,305 genes predicted present in the genome (includes both complete and partial genes, Figure 3, Table 5) of which 8.7-12.8% of the conserved genes were missing in our assembled genome across taxonomic databases (BUSCO, Figure 4).

Overall, RepeatMasker detected 65,915 repetitive elements accounting for 1.06% of the assembly, the majority of which were simple DNA repeats (Table 6). The repetitive landscape of *T. molitor* has been previously characterised based on

Table 3. Regression models of significant relationships between read number and quality metrics of eight test assemblies. Most measures of contiguity correlated positively with read numbers, and most models fit well to data, with adjusted R^2 values ranging between 0.62-0.95.

Analysis	Regression	Coefficient	t	P	adj. R^2
Assembly size – uncompressed	linear	0.26	11.53	<0.001	0.95
Assembly size – compressed	logarithmic	115.19	7.58	<0.001	0.89
Estimated genome size	exponential	7.06×10^{-141}	55.64	<0.001	0.99
Total number of scaffolds	logarithmic	-24,841	-4.92	0.003	0.77
Scaffolds ≥ 10 kb	logarithmic	2,481.8	8.86	<0.001	0.92
Max scaffold size	logarithmic	202.95	3.55	0.012	0.62
N50 scaffold	logarithmic	19.31	5.99	<0.001	0.83
N75 scaffold	logarithmic	5.07	7.86	<0.001	0.90
L50 scaffold	logarithmic	-6,383	-3.92	0.007	0.67
L75 scaffold	logarithmic	-15,622	-4.67	0.003	0.75
Ns per 100 kb	linear	1.302	6.49	<0.001	0.85

Figure 2. Effects of read number on assembly statistics. Data for eight assemblies (50, 56, 60, 75, 98, 118, 130, and 193x) were analysed with regressions. Overall, more reads resulted in larger, less fragmented assemblies at the cost of increasing ambiguity. Estimated genome size and assembly size, compressed or not, increased with the number of used reads (A). This trend was also observed in the number of scaffolds ≥ 10 kb, but not in scaffolds of all sizes (B). Maximum scaffold size (C) and scaffold N50 and N75 (D) were also positively correlated with read number. As scaffolds increased in length, L50 and L75 (E) decreased while the amount of Ns (F) they contain increased. Associate R^2 values are printed on each regression line.

Table 4. Assembly statistics for the 130x assembly. Compared to other test assemblies, the 130x assembly was optimal in quality, having the largest scaffold, the highest number of large scaffolds (≥ 10 kb), high N50 and N75, low L50 and L75, and relatively few Ns per 100 kb.¹

Assembly metrics	Value
Uncompressed assembly size (bp)	295,039,520
Compressed assembly size (bp)	201,953,383
Estimated genome size (bp)	321,280,000
Total number of scaffolds	31,391
Number of Scaffolds ≥ 10 kb	6,617
Length of largest scaffold (bp)	415,709*
Scaffold N50 (bp)	26,962*; 41,462
Scaffold N75 (bp)	8,586*
Scaffold L50	3,050*
Scaffold L75	8,049*
Contig N50 (bp)	39,478
Raw coverage (x)	103.403
Effective coverage (x)	59.8937
GC (%)	36.04
#N's per 100 kb	324.92*

¹ * means outputs computed by QUAST outputs, all other metrics computed by Supernova.

Table 5. Numbers of sequences predicted by AUGUSTUS for the 130x assembly, including the number of partial genes predicted.

Sequence type	Number of complete	Number of genes including partial genes
Gene	16,090	20,305
mRNA	16,090	20,305
CDS	16,090	20,305
Amino acid	16,090	20,305
Exon	108,412	114,393
Intron	92,322	103,191

Table 6. Types, counts, and total lengths of repetitive elements detected in the 130x assembly by RepeatMasker.

Sequence type	Number of elements ¹	Length occupied (bp)	% of sequence
DNA transposons	950	321,487	0.11
Unclassified	4	180	0
Small RNA	188	55,767	0.02
Satellites	200	71,256	0.02
Simple repeats	51,723	2,053,642	0.70
Low complexity	12,850	612,218	0.21
Total	65,915	3,114,550	1.06

¹ Most repeats fragmented by insertions or deletions have been counted as one element.

Figure 3. Size in base pairs (bp) of genes, transcripts, coding sequences, amino acid sequences, exons, and introns predicted by AUGUSTUS for the 130x assembly. AUGUSTUS was trained with annotated genes in *Tribolium castaneum* to model probability distributions for different types of sequences (e.g. coding DNA, mRNA, amino acid) and gene components (e.g. exons and introns), and apply the model to the assembly. 'Partial' bars included predictions that missed certain components based on the training data (e.g. partial genes = missing some exons). As an example, complete genes are 8,990 bp on average, with some as small as 223 bp and as large as 125,407 bp.

Figure 4. BUSCO results characterising gene content completeness of the 130x assembly. The numbers of genes in each searched database are as follow: Eukaryota = 303, Arthropoda = 1,066, Insecta = 1,658, Endopterygota = 2,442. Counting both single-copy and duplicate genes, most Arthropoda orthologs were found (89%) while Endopterygota had the fewest hits (81.3%).

the abundant presence of the 142-bp satellite sequence (Petitpierre *et al.*, 1988). RepeatMasker detected 334 instances of this satellite across 155 scaffolds, accounting for 0.02% of base pairs in the uncompressed assembly. Similarly in a BLAST analysis, the simple repeat was detected in 112 scaffolds (BLASTn overlap >90%, identity ≥69%; average mapping coverage = 58,020.2), with repeating unit ranged from one to 25 times (average = 4.11 ± 5 SD) and accounted for roughly 0.015% of the uncompressed assembly. In the scaffold with 25 repeats, the satellite sequence measured 3,350 bp or 35.7% of the scaffold length. When raw reads were mapped to the satellite sequence, 24.75% of the reads mapped, a substantial portion of reads from the genome that are associated with this repetitive unit.

The mitochondrial genome was assembled in a single scaffold with an average coverage of 18,051 and a length of 15,785 bp (72.4% A/T, 0% N) and 99.75% sequence similarity to the published mtDNA genome, containing eight gaps and 31 nucleotide variants. Amino acid sequences of the 13 mitochondrial protein-coding genes were used as query against the consensus sequence translated across all reading frames. Significant BLAST hits with E-values = 0 and without internal stop codons suggested the recovered scaffold was indeed the mtDNA genome. For three genes in our mitochondrial assembly, amino-acid polymorphisms were detected relative to the queries: cytochrome b (leucine → methionine at position 305), cytochrome c oxidase subunit III (threonine → alanine at position 173), and NADH dehydrogenase subunit 4 (valine → methionine at position 379). The mtDNA can be accessed via NCBI (accession number MT041765).

4. Discussion

We present the first linked-read assembled draft genome for the economically important insect species *T. molitor*. Several animal genomes have been assembled with this sequencing technology (Jones *et al.*, 2017; Ozerov *et al.*, 2018; Wang *et al.*, 2019), which has the advantages of producing less fragmented assemblies than standard short reads (Huddleston *et al.*, 2014; Pareek *et al.*, 2011), while avoiding the high rate of sequencing error typically associated with long-read technologies (Del Angel *et al.*, 2018; English *et al.*, 2012). As such, our genome is a more complete resource for the community when working to optimise insects as food and feed.

Assuming a complete genome with 508 Mb (Gregory *et al.*, 2007; Juan and Petitpierre, 1991), the Supernova assembler's estimates of genome size using the distribution of k-mers in raw reads suggested that sequencing only captured roughly 62–81% of the total content (Figure 2A). While this may well be the case, several factors can produce genome assemblies that are smaller than the expected sizes. For example, the repetitive landscape may obscure

the real genome size. If the 142-bp simple repeat is actually present in ~25% of the reads, then the assembly would be smaller due to reads mapping to that specific region. Based on uncompressed and compressed sizes, our 130x draft assembly captured 58% of the structure and 40% of the genetic information of the genome (Table 4). The missing portion is likely attributable in part to the well-characterised 142-bp satellite which is estimated to make up between 44 and 56% of the genome (Petitpierre *et al.*, 1988). In the final assembly, however, we were only able to capture a small part of this repetitive element which accounted for about 0.02% of the assembly size. Indeed, the successful assembly of scaffolds containing repetitive elements demonstrated the advantage of linked-reads in piecing together difficult-to-align regions. Estimated genome size was stable around 310 Mb when 26–67% of reads were utilised by the assembler, and increased to 413 Mb when all reads were used (Table 1, Figure 2A). On the other hand, independent estimates of genome size by GenomeScope computed from a range of k-mers suggested that most or all of the genome was fully sequenced including repetitive content (Table 2).

A drawback of using more reads during assembly is that more ambiguous bases tend to be introduced, and we saw greater value in assembling a genome that maximises the amount of genic information. Our selection of the 130x assembly was based on numerous statistics that suggest it was the most optimal in balancing usable information and noise. Despite missing a portion of the genome, our assembly performed well on the gene content completeness benchmark with >87% of conserved orthologs (complete or partial) detected for each taxonomic level (Figure 4). In addition, gene model prediction suggested the current assembly contains 16,090–20,305 coding loci, similar to figures found in *T. castaneum* (Richards *et al.*, 2008) and other beetles (McKenna, 2018).

Variation at the amino acid level observed between our mitochondrial sequence and the published mitochondrial genome can probably be explained in part by the history of the specimens. Ours came from a strain kept for over a decade at the National Biological Control Laboratory in Stoneville, MS, USA with ancestry in the domestic pet-food industry (Morales-Ramos *et al.*, 2019), while the published mtDNA was derived from an inbred laboratory line established in 2008 in China (Liu and Wang, 2014). These two populations likely have undergone multiple genetic bottlenecks, and have been reared in different environments with variable growing conditions and stressors. In fact, the Stoneville strain experienced a period of population contraction during a selection experiment for larger body size, which has been in effect for at least six to seven generations prior to sequencing (Morales-Ramos *et al.*, 2019). These factors may be sufficient to drive divergence and lead to amino acid substitutions.

The trade-offs between beneficial and undesirable traits have previously been a major obstacle to efficient mass rearing efforts of insects. For example, tephritid fruit flies, when mass-reared, show increased fecundity and faster developmental times, but experience trade-offs such as lower resilience to stress, and reduced longevity (Gilchrist *et al.*, 2012). Selection for larger body size in the yellow mealworm is correlated with a trade-off in early larval mortality (Morales-Ramos *et al.*, 2019). It is unknown how these traits are linked, and what the genetic basis might be for such trade-offs. With genomic tools, researchers can better understand the genetic basis of phenotypes and test hypotheses of trait linkages to resolve commercially costly trade-offs.

We present a high-quality draft genome of the yellow mealworm *T. molitor*, which is now available to researchers and industry alike, to further advance our understanding of mealworm biology. Ongoing efforts to optimise mass-rearing of the yellow mealworm are expected to be significantly facilitated by this draft genome. With this resource, we contribute to the study of genomics and evolution in beetles, but we also aim to elevate the yellow mealworm as a competitive protein source for food production and as an animal feed ingredient.

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Conflict of interest

The authors declare no competing interests.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/JIFF2019.0057>.

Supplementary material S1. Glossary of genomic sequencing and bioinformatic-based genome assembly terms.

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