Chromosome-level hybrid *de novo* genome assemblies as an attainable option for non-model organisms

Running title: Achieving *de novo* chromosome-level genome assembly

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Abstract

The emergence of third generation sequencing (3GS; long-reads) is making closer the goal of chromosome-size fragments in *de novo* genome assemblies. This allows the exploration of new and broader questions on genome evolution for a number of non-model organisms. However, long-read technologies result in higher sequencing error rates and therefore impose an elevated cost of sufficient coverage to achieve high enough quality. In this context, hybrid assemblies, combining short-reads and long-reads provide an alternative efficient and cost-effective approach to generate *de novo*, chromosome-level genome assemblies. The array of available software programs for hybrid genome assembly, sequence correction and manipulation is constantly being expanded and improved. This makes it difficult for non-experts to find efficient, fast and tractable computational solutions for genome assembly, especially in the case of non-model organisms lacking a reference genome or one from a closely related species. In this study, we review and test the most recent pipelines for hybrid assemblies, comparing the model organism *Drosophila melanogaster* to a non-model cactophilic *Drosophila*, *D. mojavensis*. We show that it is possible to achieve excellent contiguity on this non-model organism using the DBG2OLC pipeline.

Keywords: accuracy; chromosome-level; contiguity; long-read; short-read.

1. Introduction

Whole genome sequencing is a major target in evolutionary biology, because it provides the material to study how a species' genome evolves. Notably, whole genome data provides the opportunity to study recombination and large rearrangement events, differential molecular evolution across the genome, and imprints of selection throughout the genome, ultimately improving our knowledge of how species evolve and diverge (Ellegren 2014, Rudman et al., 2018). To increase our understanding of such evolutionary processes, we need to expand the range of studied organisms to non-model organisms, for which the access to well resolved genome assemblies is often lacking.

Thanks to third generation sequencing (3GS) from platforms such as PacBio (Rhoads & Au 2015) and Nanopore (Urban, Bliss, Lawrence & Gerbi 2015), *de novo* genome assemblies of non-model organisms can be obtained, but one drawback from such technologies is the high error rate. *De novo* hybrid assemblies combine long-reads and short-reads (Illumina technology; Bentley et al., 2008) to achieve high contiguity and accuracy while reducing sequencing costs through lower coverage of long-reads data (Ye, Hill, Wu, Ruan & Ma, 2016).

There is a constantly increasing panel of tools to assemble reads and polish genome assemblies. Identifying the pipeline most optimized to one's needs is one obstacle, and applying it to the actual data is another one, especially in the absence of bioinformatic expertise, since guidelines and practical implementations remain limited. In addition, many of those pipelines are not tested on non-model organisms and assume that the samples are from model organisms where extreme

inbreeding and high homozygosity is commonly feasible. In the present study, we reviewed the most recent whole genome assembly pipelines, and selected a promising pipeline relying on hybrid technology (Chakraborty, Baldwin-Brown, Long & Emerson, 2016). We tested it thoroughly with the aim of an optimized assembly, using DNA data from both *D. melanogaster* as a model species, and *D. mojavensis* from the Sonora, Mexico population as a non-model species. Ultimately, this new *D. mojavensis* assembly from Sonora will be used in a much larger upcoming genomic study using *de novo* assemblies of multiple cactophilic species and populations (Matzkin, unpublished data). We provide here an analysis of the effects of different parameters on the quality of the final assembly, assessed by a combination of universal tools (contigs length and N50 as a measure of contiguity; BUSCO score as a measure of quality and completeness (Waterhouse et al., 2017) and a reference-based tool, Quast (Gurevich, Saveliev, Vyahhi & Tesler, 2013) which compares the assembly to a reference genome. We show a significant improvement of assembly quality compared with results from Chakraborty et al. (2016) simply by tuning parameters and we provide guide parameters for assemblies with similar coverage of non-model organism DNA. Finally, we tested the pipeline on *D. mojavensis* from the Santa Catalina Island, California population using Nanopore long-read data instead of PacBio data.

2. Materials and Methods

2.1. *Drosophila mojavensis* sequencing

We used flies from the *Drosophila mojavensis* isofemale line MJ 122 collected from Guaymas, Sonora Mexico in 1998, thereafter SON. Prior to DNA extraction the flies were raised on banana-

molasses medium (Coleman, Benowitz, Jost & Matzkin, 2018) with 125 µg/ml ampicillin and 12.5 µg/ml tetracycline to reduce bacteria contamination of the sequencing data. The sequencing methods for short-read data (paired ends and mate pairs) have been described in Allan & Matzkin (2019). Sequencing technologies and coverage for the different data sets are summarized in Table 1.

2.1.2. DNA extraction for PacBio Sequencing – Protocol optimization

Due to the long-read potential of PacBio Sequencing Systems, extra care must be taken during extraction to produce high molecule weight DNA. Attempts at using both QIAGEN DNeasy Blood & Tissue Kit and QIAGEN MagAttract HMW DNA Kit failed to produce sufficiently long strands of DNA. As such, a chloroform-based extraction method was used. This relatively simple method is low cost and the only specialized equipment needed is a refrigerated centrifuge. To consistently recover enough DNA for two PacBio libraries, 150 flies of each sex were used for extraction. Flies were starved for two hours in groups of 50 per vial and then frozen at -80° C in 1.5 mL tubes. A lysis solution containing Tris HCl buffer 0.1M pH 8.0, EDTA 0.1 M pH 8.0, and 1% SDS was prepared and stored at room temperature to prevent the SDS from precipitating. While on ice, 500 µL of lysis solution was added to each tube of flies, followed by 2.5 µL of QIAGEN Proteinase K to reduce DNA degradation. Using a plastic pestle, flies in each tube were hand-homogenized by gently grinding them. Hand homogenization resulted in slightly lower amounts of DNA recovered, however the size of DNA fragments were longer compared to when using a battery-operated pestle motor to homogenize. The mixture was incubated at 65 °C for 30 min with gentle mixing halfway through. To further reduce DNA fragmentation, tubes were cooled to 37 °C for three minutes and another 2.5 µL of QIAGEN Proteinase K was added. Tubes were incubated for

an additional 30 min at 37 °C. After incubation, 70 µL of 4 M potassium acetate was added, mixed by inversion, and then placed on ice to incubate for 30 minutes. In a 4 °C Eppendorf 5920R centrifuge, the tubes were spun for 30 min at 18,000 rcf to pull debris to the bottom of the tubes. For each tube, the supernatant was transferred to new tubes avoiding as much debris as possible. One volume of chloroform:isoamyl alcohol 24:1 was added to each tube and gently inverted 40 times, and then centrifuged at 4 °C for 5 min at 10,500 rcf. The upper phase was transferred to a new tube while being careful to not disturb the interface. The DNA was precipitated by adding 350 µL of 2-propanol and gently inverting the tube. At this point visible threads of DNA were apparent. To pellet the precipitate, the tubes were centrifuged at 4 °C for 5 min at 10,500 rcf. The supernatant was discarded and the pellet was washed with 1 mL of room-temperature 70% ethanol. The tube was inverted to insure washing of the pellet and tube. A final 4 °C centrifugation for 2 min at 10,500 rcf was performed. The ethanol was removed with a pipettor as completely as possible and the pellet dried for 10-15 min in a fume hood. 30 µL of Tris-EDTA pH 8.0 was added to each tube to resuspend the DNA. While the pellet normally goes into solution relatively easily, it can be placed at 4 °C overnight to insure resuspension. The six tubes were then combined to a single tube and 3 µL of QIAGEN RNaseA was added and incubated for 30 min at 37 °C. The DNA was delivered as this resuspended solution for PacBio sequencing.

2.1.3. PacBio Sequencing

PacBio sequencing was performed at the Arizona Genomics Institute (Tucson, AZ, U.S.A.). DNA was sized in a 1% agarose pulsed field gel electrophoresed at 1-50 sec linear ramp, 6 volts/cm, 14 °C in 0.5X TBE buffer for 20 hours (BioRad). The marker used was a lambda ladder Midrange PFG I (New England Biolabs). The resultant DNA smear had a large mass in the 35-65 kb range

(Fig. 1). DNA purity was verified using a NanoDrop One Microvolume UV Spectrophotometer with ratios 260/280 and 260/230 over 1.8. Quantity (150 ng/μL in 180 μL = 27 μg) was determined by a Qubit Fluorometer (Life Technologies), and was consistently lower than that measured with the Nanodrop. PacBio sequencing libraries were prepared from 6 μg starting material each, following manufacturers protocol for a 20 Kb Template Preparation Using BluePippin™ Size-Selection System (www.pacb.com). The library was size-selected, on a BluePippin, at 20 kb using high pass with S1 Marker (Sage Sciences). The final library was damage-repaired, bead-purified and quantified. Sequencing was performed on a PacBio Sequel instrument following manufacturer's instructions. The sequencing primer annealed was v3, the sequencing kit was v2.1. Two libraries were loaded on two separate SMRT cells with magbeads at concentrations of 25 pmol and 35 pmol, respectively. Sequencing was carried out for collection of 10 hr movies on 1 M SMRT cells.

2.2. *Drosophila melanogaster* and *D. mojavensis* from Catalina public sequencing data

To generate the *D. melanogaster* assembly (hereafter, Dmel), PacBio data was retrieved from the NCBI Short-read Archive SRX499318 (Kim et al., 2014). This data set contained 42 PacBio RS II SMRT cells from male *D. melanogaster* ISO1 flies. We used data from 20 randomly selected cells only to obtain a coverage similar to our data sets (cell numbers SRR1204085, SRR1204088, SRR1204451, SRR1204466, SRR1204467, SRR1204469, SRR1204471, SRR1204472, SRR1204473, SRR1204481, SRR1204482, SRR1204485, SRR1204486, SRR1204615, SRR1204617, SRR1204690, SRR1204691, SRR1204692, SRR1204693, and SRR1204696). We used the SMRT Illumina HiSeq 2000 100 bp paired-end data from male *D. melanogaster* ISO1

flies, which was retrieved from the European Nucleotide Archive ERX645969 (Miller, Smith, Hawley & Bergmann, 2013).

For the *D. mojavensis* assembly from the Santa Catalina Island, California population (hereafter, CAT), Nanopore sequencing data was kindly provided by Miller, Staber, Zeitlinger & Hawley (2018). Short-read Illumina data was retrieved from the NCBI Short-read Archive SRR6425997 (Miller et al., 2018).

2.3. Computing resources

All the programs were run on the UA Research Computing High Performance Computing (HPC) at the University of Arizona. The cluster used is composed of 28 core processors with 168 gb RAM per node, and is run via a PBS-Pro grid system. All the programs used were installed under a user python virtual environment (pip). The majority of the programs used are available as Bioconda packages for easy installation in non-cluster environments (Grünings et al., 2018). They are also provided as Docker containers through Bioconda which can be run through *Singularity* (<https://sylabs.io/>) on cluster systems. All command lines are provided in Appendix.

2.4. Assembly pipelines

2.4.1. DBG2OLC Pipeline

The DBG2OLC Pipeline is composed of three main steps: (i) the hybrid assembly via the DBG2OLC program, (ii) the long-read assembly only, and (iii) the merging of those two assemblies (Fig. 2).

(i) Hybrid assembly

DBG2OLC uses contigs from a short-read assembly and maps them to the raw long-reads, which are then compressed into the list of the short-read's contig identifiers (Ye et al., 2016). A best overlap graph is constructed from those compressed long-reads before uncompressing them into a consensus sequence. This method is both highly accurate and extremely fast (Ye et al., 2016). Then, the consensus contigs, or backbones are corrected using *Sparc* (Ye & Ma, 2016). *Sparc* builds a sparse k-mer graph (k-mers in different positions are treated independently) using the contigs identifiers' list associated with each raw long-read. All short-read contigs are then aligned to their associated long-read using the *Blasr* aligner from the PacBio SMRT toolkit (SMRT Link v4.0.0), previously *Pbdagcon*, which is the most time-consuming step. *Sparc* finally uses these alignments to refine the graph and create a polished consensus sequence. In the present study, we tested two competing short-read assemblers, *SparseAssembler* (provided with the *DBG2OLC* installation package) (Ye, Ma, Cannon, Pop & Yu, 2012), and *Platanus* version 1.2.4 (Kajitani et al., 2014). We used the March 2019 version of *DBG2OLC* (Ye et al., 2016), the January 2015 version of *Sparc* (Ye & Ma, 2016), and *Blasr* 5.3.5 (b30da0) (SMRT Link v4.0.0). Note that we began working with an older version of *Blasr* which was significantly slower and led to slightly different results. For this reason, and because programs often include third party packages, it is important to keep track of each version used and physically separate the repositories, so the SMRT toolkit was installed in an independent directory with no direct link to the user bin, except for the *Blasr* program. We modified the `split_and_run_sparc.sh` script available from the *Sparc* Github repository so as to call the `split_reads_by_backbone.py` script externally (Appendix I), and to set the number of chores used by *Blasr* from the command line. This way, it is easier to rerun the time-

consuming Sparc step in case of crash from where it stopped, and after moving the already corrected backbones into another directory.

The hybrid assembly was then polished using the PacBio tool in the SMRT toolkit (SMRT Link v4.0.0). The version of the PacBio correction tool is frequently updated along with chemistry technology of PacBio sequencing, therefore the version *Quiver* (v2.1.0) was used for *D. melanogaster* (sequenced in 2014 on a PacBio RS II system; Kim et al., 2014), and the version *Arrow* (v2.1.0) was used for *D. mojavensis* (sequenced in 2017 on a PacBio Sequel system installed with SMRT Link v4.0.0, see above). For simplicity, we will thereafter refer to that step simply as '*Quiver*'. *Quiver* aligns the raw PacBio reads to the assembled and corrected contigs output by *Sparc*, and uses a consensus caller to polish them (Chin et al., 2013). Lastly, the hybrid assembly was polished using *Pilon* v1.22 (Walker et al., 2014). *Pilon* uses raw short-reads aligned to the assembly with the *Bowtie2* aligner version 2.2.9 (Langmead, Trapnell, Pop & Salzberg, 2009), to first find and correct SNPs and small indels (base error consensus), and secondly local misassemblies (alignment discrepancies scan) that are reassembled using paired ends and mate pairs (if provided). Parameters were optimized at each step: (a) choice of the short-read assembler (*Platanus* vs. *SparseAssembler* with kmer-size 39 or 53); (b) *DBG2OLC* parameters, based on recommended optimization ranges (Ye et al., 2016): MinOverlap in [20; 150]; AdaptiveTh in [0.002; 0.02]; KmerCovTh in [2; 10] and MinLen in [200; 2,000]; default values were otherwise used ($k = 17$; LD1 = 0); (c) ContigTh 0 (default) vs. 1 (recommended for > 100x PacBio coverage only); and (d) *Sparc* one vs. two iterations. These parameters are summarized in Table 2.

(ii) Long-read assembly only

The long-read assembly was created using *Canu* v1.5 (Koren et al., 2017), which significantly outperforms its older version *Celera Assembler* (PbcR) used in Chakraborty et al. (2016) as well as other assemblers, notably by using an adaptive kmer weighting which both improves the efficiency and the quality of the assembly of highly repetitive genomic regions. We tested two parameters for the `correctedErrorRate`: 0.039 vs. 0.055 (low end and middle value of recommended range, Table 3, Koren et al., 2017). Note that this adjustment is limited by coverage, and thus intrinsic to the analyzed data set. We ran the three *Canu* steps (correction, trimming and assembly) separately using the options `-correct`, `-trim` and `-assemble` (see Appendix) to optimize the assembly step without running again the first two steps. Similarly to the hybrid assembly, the long-read only assembly was polished using both *Quiver* and *Pilon*.

(iii) Assembly merging

The hybrid assembly and long-read only assembly were merged after polishing using the *Quickmerge* tool v0.2 (Chakraborty et al. 2016). *Quickmerge* uses *MUMmer* (v 3.0) (Kurtz et al. 2004) to align the two assemblies and find the unique best alignment (using the `-delta-filter` option in *MUMmer*). *Quickmerge* then identifies high confidence overlaps between the two assemblies to find seed contigs (i.e., contigs that can be extended at both ends). Finally, it merges the overlapping contigs using sequences from the reference (donor assembly) into the query (acceptor assembly). The optimization consisted of trying both the hybrid and long-read assemblies as reference vs. query, and varying the `l` and `lm` cutoff parameters in *Quickmerge* (Table 3). Lastly, the merged assembly was polished using *Quiver* and *Pilon*.

2.4.2. Test of the DBG2OLC pipeline with Nanopore long-reads

We ran the DBG2OLC pipeline on CAT sequencing data using Nanopore reads instead of PacBio reads for the long-read only assembly, the hybrid assembly, and the polishing steps. We used the optimal parameter set (2.4.1; P6), except for *Canu*, for which we had to increase the `correctedErrorRate` to 0.055 to recover 97% of the genome, while we could recover only 51% using a `correctedErrorRate` of 0.039. Instead of *Quiver*, we used *Nanopolish* version 0.11.0 (Simpson et al., 2017). Similar to *Quiver* and *Pilon*, raw Nanopore reads were first aligned to the target assembly using the *Bwa* aligner version 0.7.17 (Li & Durbin, 2010). *Nanopolish* then generates an improved consensus sequence.

2.4.3. Alternative Pipelines

DBG2OLC was identified as the only pipeline, among the most recently published assemblers, allowing the assembly of long-reads prior to correction. Alternatively, long-reads may be corrected prior to assembly, as is the case in the *Canu* pipeline. Other possible correction tools include *LSCplus* (Hu, Sun & Sun, 2016), a modified version of the *MHAP* tool (indexing kmers used to build the assembly graph in *Celera*; (Carvalho, Dupim & Goldstein, 2016), *HALC* (Bao & Lan, 2017), and *FMLRC* (Holt, Wang, Jones & McMillan, 2016), and most recently (not tested here) *MECAT* (Xiao et al., 2017) and *Jabba* (Miclote et al., 2016). The *LSCplus* package was not available at the time of our study, and we were therefore not able to assess its efficiency. The modified *MHAP* tool was implemented in *Celera* only (the older version of *Canu*), which thus yielded poor results in term of assembly contiguity, and this solution was abandoned. Note however that some aspects of kmers indexing as proposed in the modified *MHAP* tool have now been implemented in *Canu* (Koren et al., 2017), and were therefore implicitly used in our *DBG2OLC* Pipeline. *FMLRC*, although it correctly performed the long-read correction, proved to

be non-compatible with *Canu* (Holt et al., 2016). Therefore, this alternative was abandoned as well.

HALC corrects long-reads by (i) aligning them to the contigs from a short-read assembly, (ii) constructing a graph from this alignment, and (iii) finding the best path in the graph to correct each long-read. It relies on *Blasr* (SMRT Link v4.0.0) for the alignment and on *LoRDEC* (Salmela & Rivals 2014) for the correction. We used the one version of *HALC* available, *Blasr* 5.3.5 and *LoRDEC* 0.6 with the *GATB* library 1.0.6. After read correction with *HALC*, we ran *Canu* (-assemble option) with a correctedErrorRate = 0.039. The contiguity of the assembly was orders of magnitude worse than when using the *Canu* correction tool and the same assembly parameters (*HALC* correction: N50 = 488,850; total length = 138,021,997; max length = 2874227; *Canu* pipeline: N50 = 10,9990,654; total length = 151,043,692; max length = 25,950,142). This might have been improved by parameter optimization of both the *HALC* correction step and the assembly step with the *Canu* assembler, but due to the strong difference in contiguity we chose to not utilize *HALC*. Therefore, we focused on optimizing the *DBG2OLC* pipeline only.

2.5. Assembly quality check

Comparisons between assemblies and quality assessment were performed based on assembly statistics from *Quast* version 4.6.2 (Gurevich et al., 2013) by comparing each assembly to a reference genome to estimate the number of global and local misassemblies as well as the number of mismatches and indels. For both general statistics (number of fragments, N50) and error rates (presented in Tables 4-6), we used contigs longer than 400 bp only, so as to run the program faster. We also calculated *BUSCO* scores using the diptera (odb9) set of Benchmarking Universal Single-

Copy Orthologs (Waterhouse et al. 2017). We used the reference genomes FB2017_01 and FB2015_02 (Consortium DG, 2007) released on FlyBase (Thurmond et al., 2019) for Dmel and CAT, respectively. For SON, we used a template assembly constructed based on the Catalina reference genome (Allan & Matzkin, 2019). For each data set, we extracted only the fragments that have been previously designated to chromosomes (i.e., for Dmel, the four chromosomes; and for SON and CAT the 39 biggest scaffolds), so as to run quality assessment faster. We are aware that using a template assembly as a reference for SON may introduce biases especially in terms of number of misassemblies, due to the evolutionary history of the *D. mojavensis* populations (Matzkin, 2014) therefore the results must be considered carefully. However, this provides a valid guide to make relative comparisons between assemblies created here. *Quast* relies on *MUMmer* v3.23 (nucmer aligner v3.1; Kurtz et al., 2004) to align the assembly to the reference genome, and includes metrics and methods from the *GAGE* assessment tool (Salzberg et al., 2012) and other tools.

Finally, assemblies were aligned to their reference genome using MUMmer4 (Marçais et al., 2018) and plotted against the reference genome using Dot (<https://github.com/dnanexus/dot>).

2.6. Test of the *DBG2OLC* pipeline with Nanopore long-reads

We tested the *DBG2OLC* pipeline on *D. mojavensis* population Catalina using Nanopore long-reads instead of PacBio long-reads with parameters optimized for SON: *Platanus* short-read assembler; *DBG2OLC* parameters MinOverlap 150; AdaptiveTh 0.020; KmerCov 2; MinLen 200; number of *Sparc* iterations 2 or 3 (both tested: P6r” vs. P6fr”. For the *Canu* assembly, we used the correctedErrorRate = 0.055 since lower rates resulted in incomplete genome (0.039:

73.7 %; 0.045: 86.7 %; 0.055: 93.8 %). For the merged assembly, we used parameters as optimized for SON: the hybrid assembly as query; $l = 1$ Mb and $lm = 10,000$ bp (Table 3).

3. Results and Discussion

3.1. DNA preparation

The custom chloroform extraction led to a remarkable increase in the sizes of DNA fragments (light band between 30 and 120 Kb, right panel, Fig. 1) compared with standard extraction kits (left and middle panels, Fig. 1) for which the majority of fragments were shorter than 30 Kb. Long fragments in DNA libraries significantly increase DNA quantity output by PacBio sequencing (www.pacb.com).

3.2. Optimization of the DBG2OLC Pipeline

3.2.1. Short-read assembler

Platanus and *SparseAssembler* with a kmer size of 53 bp resulted in very similar assemblies; *SparseAssembler* with a kmer size of 39 bp led to reduced contiguity; and applying two successive rounds of *SparseAssembler* 53 bp-Kmer size did not improve the short-read assembly. In the final merged assemblies, the use of *SparseAssembler* always led to slight decrease in contiguity (comparing P3 to S3 and P6 to S6 for both *Dmel* and SON, Table 4). *SparseAssembler* slightly reduced error rates but also BUSCO scores for *Dmel*, with limited effects for SON (Fig. 3B). We also observed that differences in P6 and S6 for SON mainly resided in highly repetitive regions. Since *Platanus* is especially recommended for genomes with high level of heterozygosity, which

are more likely in non-model organisms, we used *Platanus* for all the subsequent assemblies in this study.

3.2.2. DBG2OLC parameters

We varied the DBG2OLC parameters **MinOverlap**, **AdaptiveTh**, **KmerCovTh** and **MinLen** to simultaneously optimize the contiguity and quality of the final assembly. Misassemblies created during the first steps of the hybrid assemblies were overall not resolved later, which makes that step key to the optimization. P0 corresponds to the reference set of parameters used in Chakraborty et al. (2016). Note that P0 has similar contiguity as shown in Chakraborty et al. (2016) for the assembly with ~100x coverage although with lower error rates, indicating that our analysis using their parameter set resulted in a very similar result.

MinOverlap had a major effect on final assemblies, with a major improvement of contiguity (reduced number of fragments, increased N50, increased length of longest fragment; Fig. 3A,C) and of accuracy (reduced number of global and local misassemblies, reduced number of mismatches and indels; Fig. 3B, 3D) as seen in the P0 vs. P3 and P1 vs. P6 comparisons. This came at a cost of a slight decrease in BUSCO score for Dmel but not SON. Only an increase of **MinOverlap** up to 150 (the maximum recommended value for more than 50x coverage of PacBio reads) led to an optimal lower number of misassemblies (P2 vs. P6).

AdaptiveTh had little influence, except when **MinOverlap** was kept low: it decreased contiguity and accuracy (P2 vs. P0). For assemblies with high **MinOverlap**, we found that P3 was less fragmented than P4, P5 or P6 for SON and P4 was the least fragmented for Dmel. P6 was the best

compromise between contiguity and accuracy for SON, with the highest BUSCO score. P4 was the best compromise and with the highest BUSCO score for Dmel. Although coverage in both Illumina short-reads and PacBio long-reads was lower in SON than Dmel (Table 1), the quality of PacBio long-reads was higher (longer reads thanks to DNA extraction protocol, and more recent PacBio technology), which might have facilitated the better results with the more stringent AdaptiveTh. Therefore, for PacBio data sequenced with the newest technology and coverage higher than 50x, we recommend to use the most stringent value for AdaptiveTh, of 0.020.

High KmerCovTh values resulted in major global misassemblies in SON (assessed with Mummer plots, not shown), with the largest fragment longer than the theoretical longest fragment in the Reference assembly (P4y vs. P4 and P6y,x vs. P6). It also caused a slight increase in error rates and a slight decrease in BUSCO score. In Dmel, no major global misassembly was detected, however error rates were higher and BUSCO scores slightly lower. We recommend to use KmerCovTh = 2, especially when using high AdaptiveTh. Using ContigTh = 1 had similar effects (major global misassemblies; P6g vs. P6 for SON) than high KmerCovTh values, so we recommend not using it.

Increased MinLen from 200 to 2,000 resulted in a slight increase in contiguity for both Dmel and SON (PX vs. PXa). Error rates and BUSCO scores were not notably different, unless MinLen was increased up to 5,000 in which too many reads were parsed out, leading to higher error rates. Due to the absence of major effects and according to DBG2OLC recommendations (Ye et al. 2016), we recommend to not increase MinLen above 200, because there is a risk to parse out under-represented genome regions.

Increasing the number of *Sparc* iterations from 2 to 3 allowed a higher contiguity of large fragments, although with little effect on overall statistics. Increasing *Sparc* iterations is supposed to reduce the number of chimeras. Since it had no negative effect, we recommend using three iterations.

Based on our tests, we recommend the following DBG2OLC parameters, for 50x or more of PacBio reads from recent technology: `MinOverlap 150`; `AdaptiveTh 0.020`; `KmerCov 2`; `MinLen 200`; number of *Sparc* iterations 3. These parameters were used throughout the next optimization step (except `AdaptiveTh 0.010` for *Dmel*).

3.2.3. *Canu* parameters

Increasing `correctedErrorRate` from 0.039 to 0.055 slightly increased contiguity of the merged assembly (P4q vs. P4r for *Dmel* and P6q vs. P6r for SON; Table 5). However, it also increased error rates overall, and decreased the BUSCO complete genes score, especially for SON. We therefore recommend to keep the *Canu* `correctedErrorRate` as low as possible, to 0.039.

3.2.4. *Quickmerge* parameters

Parameters used in *Quickmerge* are shown in Table 3, and results in Table 5. Using the long-read assembly as the Query assembly resulted in a strong decrease in contiguity compared with the opposite (P4s vs. P4q for *Dmel* and P6s vs. P6q for SON). It also considerably increased error rates for both species, and slightly decreased BUSCO complete gene score for SON. Similarly to Chakraborty et al. (2016), we therefore recommend to use the hybrid assembly as query.

394

395 We also tested the impact of the *l* and *lm* parameters. Using low *lm* with high *l* resulted in identical
396 assemblies (P4p vs. P4 for Dmel and P6p vs. P6 for SON) since backbones were already parsed
397 out due to high *lm*. Also, using *lm* = N50 or *l* = N50/2 resulted in identical assemblies for Dmel.
398 Otherwise, decreasing *l* resulted in lower number of fragments but higher error rates. However,
399 using a too high *lm* value would prevent smaller fragments from being merged, this is why we
400 recommend an intermediate value of 1 Mb (P6fn”).

401

402 3.2.5. *Polishing*

403 Polishing with both Quiver/Arrow and Pilon did not affect the contiguity (number of fragments,
404 N50, and largest fragment; Table 6, Fig. 3) for either species. Conversely, it significantly reduced
405 the number of indels on hybrid, long-read and merged assemblies. The number of mismatches was
406 also reduced to a lesser extent. One drawback was the increase in number of misassemblies, except
407 for the merged assembly. Finally, polishing increased the BUSCO score, especially on the hybrid
408 assembly.

409

410 3.3. Test of the *DBG2OLC* pipeline with Nanopore long-reads

411 Compared with Miller et al. (2018), the CAT merged assembly was more contiguous and with a
412 higher BUSCO score, but with higher error rates (Table 5, P6r” vs. Ref. Miller), likely due to the
413 multiple polishing steps performed by Miller et al. (2018). Compared with SON, CAT assemblies
414 were less contiguous but with higher BUSCO scores (CAT-P6r” vs. SON-P6n” and CAT-P6fr” vs.
415 SON-P6fn”). These results show that the *DBG2OLC* pipeline outperforms other ones in terms of
416 contiguity using either PacBio or Nanopore long-read data.

3.4. Benefits of using the *DBG2OLC* pipeline and demonstration of effectiveness on a non-model species

By merging hybrid and long-read only assemblies, we considerably increased the contiguity compared with that of the hybrid assembly or the long-read only assembly (Table 6, Fig. 3), as shown in Chakraborty et al. (2016). Also, error rates were lower than the long-read assembly, especially for *Dmel*. To obtain such low error rates with long-read data only, a higher coverage would have been necessary representing a significant increase in sequencing cost (discussed in Chakraborty et al., 2016). For this study the *D. mojavensis* Illumina sequencing was performed in 2011, if using current sequencing core prices it will total ~ \$178 (PE 150 HiSeq lane ~\$1,300 [only 1/12th of a lane needed for the 160 Mb *D. mojavensis* genome]; quality control of library \$15; library preparation \$50-\$400 [depending if done in-house or by a core]). The PacBio sequencing was performed using a Sequel system, totaling \$3,190 (library preparation \$495 × 2 libraries; SMRT cells \$1,100 × 2). Given the recent release of PacBio's Sequel II system the cost for a similar amount of long-reads would be approximately ~\$740 (library preparation ~\$450, SMRT cell \$1,750 [would only need 1/6th of a cell for *D. mojavensis*]), therefore the *de novo* assembly described in this study could be built for less than \$1,000.

One major improvement of the merged assembly (P6fn") in SON is that the 2q⁵ inversion in the Müller element D (described in Ruiz, Heed & Wasserman, 1990) is now resolved, with the two breakpoints clearly bridging the three chromosome parts (Fig. 4). This was not the case in the hybrid assembly or the long-read only assembly (not shown). The Müller elements B, D and E in our merged assembly P6fn" are in one piece and correspond to 99.24%, 99.11% and 96.67%

respectively of the corresponding chromosomes in the CAT reference genome. The Müller element C was composed of three pieces in P6fn” accounting for 99.94% of the length of the Müller element C in the CAT reference, and the Müller element A was more fragmented, as is also the case in the CAT reference genome and all fragment lengths summed up to 94.77% of the total size in the AT reference. However, in the CAT reference genome, D was in two fragments that were joint in our assembly.

4. Conclusion

In the not too distant past genomic analysis was limited to just a set of a few model laboratory species. Although this has led to unprecedented advances in our understanding of genetics and genomics, in many instances such studies lacked an ecological context. Genome assemblies of non-model species tended to be more fragmented or tended to be built using a genome from a related model species, which is problematic if interested in trait mapping or genome structure evolution. Current sequencing and computational advancements have liberated our dependence on classical laboratory model species. Here we have outlined a widely applicable computational pipeline and sets of parameters to facilitate the construction of chromosome or nearly-chromosome level genomic assemblies in a non-model species. Overall, our PacBio merged assembly performed better than using Nanopore reads, but more work is still needed to assess any differences across multiple species, especially with newer advances to the sequencing platforms.

5. Acknowledgments

We would like to thank Rod Wing, David Kudrna and Jayson Talag at the Arizona Genomics Institute for their assistance in the PacBio sequencing. This work was supported with funding from the National Science Foundation (IOS-1557697) to LMM and the University of Arizona to LMM.

6. References

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7. Data accessibility

Raw reads are available at NCBI's SRA (<https://www.ncbi.nlm.nih.gov/sra>) under accession number XXXXXX.

8. Author contribution

568 CCJ, CWA and LMM reviewed the literature and selected the pipeline. CWA performed DNA
569 extraction. CCJ performed the bioinformatics work with the help of CWA. CCJ wrote the
570 manuscript with contributions from LMM and CWA.

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Tables

Table 1. Sequencing technology and coverage of each data set.

Organism	Genome size	Sequencing technology	Data output	Coverage
<i>Drosophila melanogaster</i>	~ 140 Mb	<u>Illumina</u> HiSeq 2000	16.8 Gb ^a	120x
		<u>PacBio</u> RS II	16.1 Gb ^b	115x
<i>Drosophila mojavensis</i> (Sonora)	~ 160 Mb	<u>Illumina</u> HiSeq 2500 100 bp paired ends	10.1 Gb ^a	63x
		2,500 bp mate pairs	5.9 Gb ^c	37x
		<u>PacBio</u> Sequel	10.4 Gb ^b	65x
<i>Drosophila mojavensis</i> (Catalina)	~ 160 Mb	<u>Illumina</u> NextSeq 500 150 bp paired ends	9.1 Gb ^a	55x
		Oxford <u>Nanopore</u> MinION	15.2 Gb	95x

^a calculated as the total number of bases in data files after trimming from *Platanus_trim*.

^b calculated as the total number of bases in data files obtained from converting .bax.h5 files into .bam files, and converting the obtained subreads.bam files into fasta files, with no specific trimming

^c calculated as the total number of bases in data files after trimming from *Platanus_internal_trim*.

582 **Table 2. *DBG2OLC* parameters.**

Assembly	Assembler	MinOverlap	AdaptiveTh	MinLen	KmerCovTh	Number of <i>Sparc</i> iterations	ContigTh
P0	<i>Platanus</i>	20	0.002	200	2	2	1
P1	<i>Platanus</i>	20	0.020	200	2	2	1
P2	<i>Platanus</i>	100	0.020	200	2	2	1
P3	<i>Platanus</i>	150	0.002	200	2	2	1
S3	<i>SparseAssembler</i>	150	0.002	200	2	2	1
P4	<i>Platanus</i>	150	0.010	200	2	2	1
P4f	<i>Platanus</i>	150	0.010	200	2	3	1
P4g	<i>Platanus</i>	150	0.010	200	2	3	2
P5	<i>Platanus</i>	150	0.015	200	2	2	1
P6	<i>Platanus</i>	150	0.020	200	2	2	1
P6f	<i>Platanus</i>	150	0.020	200	2	3	1
P6g	<i>Platanus</i>	150	0.020	200	2	3	2
S6	<i>SparseAssembler</i>	150	0.010	200	2	2	1
P4a	<i>Platanus</i>	150	0.010	2000	2	2	1
P5a	<i>Platanus</i>	150	0.015	2000	2	2	1
P6a	<i>Platanus</i>	150	0.020	2000	2	2	1
P6b	<i>Platanus</i>	150	0.020	5000	2	2	1
P4y	<i>Platanus</i>	150	0.010	200	10	2	1
P6y	<i>Platanus</i>	150	0.020	200	10	2	1
P6x	<i>Platanus</i>	150	0.020	200	5	2	1

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Table 3. *Canu* and *Quickmerge* parameters.

Species	Assembly	<i>Canu</i> correctedErrorRate	Merge parameters		
			Query assembly	l (bp)	lm (bp)
Dmel	P4	0.039	Hybrid	N50: 10,000,000	10,000
	P4f	0.039	Hybrid	N50: 10,000,000	10,000
	P4n	0.039	Hybrid	N50/2: 5,000,000	10,000
	P4o	0.039	Hybrid	N50/4: 2,500,000	10,000
	P4o''	0.039	Hybrid	1,000,000	10,000
	P4fo''	0.039	Hybrid	1,000,000	10,000
	P4p	0.039	Hybrid	N50: 10,000,000	0

	P4q	0.039	Hybrid	0	0
	P4r	0.055	Hybrid	0	0
	P4s	0.039	Long-read	0	0
SON	P6	0.039	Hybrid	N50: 2,600,000	10,000
	P6f	0.039	Hybrid	N50: 2,600,000	10,000
	P6n	0.039	Hybrid	N50/2: 1,300,000	10,000
	P6n''	0.039	Hybrid	1,000,000	10,000
	P6fn''	0.039	Hybrid	1,000,000	10,000
	P6o	0.039	Hybrid	N50/4: 0,650,000	10,000
	P6p	0.039	Hybrid	N50: 2,600,000	0
	P6q	0.039	Hybrid	0	0
	P6r	0.055	Hybrid	0	0
	P6s	0.039	Long-read	0	0
CAT	P6r''	0.055	Hybrid	1,000,000	10,000
	P6fr''	0.055	Hybrid	1,000,000	10,000

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587 **Table 4. *DBG2OLC* parameter optimization: contiguity and accuracy.** Assemblies refer to parameter sets defined in Table 2.

Species	Assembly	# Fragments	N50 (bp)	Largest fragment size (bp)	# global misassemblies	# local misassemblies	# Mismatches per 100 Kb	# Indels (per 100 Kb)	BUSCO score (%)		
									complete genes	fragmented genes	missing genes
Dmel	P0	792	16,084,532	25,835,722	2,356	1,001	12.62	9.51	98.11	0.57	1.32
	P1	900	16,023,660	24,892,400	2,656	1,027	11.82	9.55	97.32	0.61	2.07
	P2	292	21,449,278	24,867,057	1,076	371	10.27	7.35	97.07	0.64	2.07
	P3	282	19,409,490	25,811,113	890	370	10.68	6.57	97.21	0.50	2.29
	S3	314	19,674,671	24,895,732	771	312	10.03	7.03	95.64	0.79	3.57
	P4	266	21,413,354	25,775,485	623	267	8.66	6.49	98.75	0.57	0.68
	P4f	267	21,413,185	25,776,014	919	318	10.55	6.73	98.75	0.57	0.68
	P4g	227	21,412,816	25,796,604	667	263	10.05	6.47	98.39	0.57	1.04
	P5	268	19,684,947	25,810,399	882	323	10.19	6.95	97.11	0.57	2.29
	P6	261	21,455,994	24,861,269	801	289	10.65	7.02	97.11	0.64	2.25
	S6	290	19,674,784	24,911,291	726	331	10.19	6.84	95.39	0.82	3.79
	P4a	267	21,414,759	25,777,026	920	338	10.79	6.94	98.75	0.57	0.68
	P5a	270	19,684,947	25,810,399	892	323	10.19	6.95	97.11	0.61	2.29
	P6a	258	21,455,918	24,861,450	553	282	9.11	6.36	97.11	0.64	2.25
	P6b	259	21,455,964	24,861,284	785	295	10.37	6.90	97.11	0.64	2.25
	P4y	239	21,413,104	25,789,761	633	304	10.43	6.83	98.71	0.57	0.71
	P6x	257	21,450,514	24,861,357	777	324	10.64	7.08	97.11	0.64	2.25
	P6y	242	21,455,993	24,861,357	610	298	9.15	6.35	97.03	0.64	2.32
	Ref. genome	7	25,286,936	32,079,331	NA	NA	NA	NA	98.68	0.75	0.57
SON	Ref. Chakraborty et al. (P0)	NA	~23 Mb	NA	~5,500	~3,300	~18	130	NA	NA	NA
	P0	348	28,767,831	34,246,767	8,244	10,064	328.42	265.12	98.39	0.86	0.75
	P1	495	21,851,486	34,252,641	9,066	10,330	329.18	266.49	98.39	0.82	0.79
	P2	132	27,067,002	33,145,725	7,351	9,756	326.40	264.27	98.25	0.96	0.79

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P3	80	27,092,095	34,236,744	7,189	9,670	325.21	263.71	98.25	1.00	0.75
S3	90	18,984,317	34,265,435	7,214	9,687	324.97	262.72	98.36	0.93	0.71
P4	92	27,081,921	33,130,663	7,166	9,688	324.99	264.24	98.21	1.04	0.75
P5	97	27,073,333	34,231,430	7,327	9,782	324.58	263.90	98.36	0.89	0.75
P6	104	27,074,084	33,145,389	7,168	9,753	324.69	264.16	98.39	0.93	0.68
P6f	103	27,125,966	33,144,936	7,173	9,760	324.80	264.49	98.36	0.96	0.68
P6g	80	27,068,405	37,117,163	7,151	9,759	324.58	267.38	98.39	0.96	0.64
S6	119	11,627,144	33,084,645	7,280	9,737	324.83	263.96	98.25	1.00	0.75
P4a	88	27,072,608	34,217,177	7,255	9,736	324.67	263.74	98.32	0.89	0.79
P5a	97	27,081,858	34,230,744	7,391	9,750	324.72	264.10	98.36	0.96	0.68
P6a	104	27,124,465	33,144,017	7,179	9,761	325.07	264.57	98.43	0.89	0.68
P6b	98	27,089,768	34,232,191	7,237	9,755	324.76	264.37	98.39	0.93	0.68
P4y	98	27,058,011	37,135,015	7,391	9,798	324.25	267.26	98.29	1.04	0.68
P6x	99	27,066,010	33,076,049	7,211	9,740	324.72	263.24	98.32	0.89	0.79
P6y	100	27,061,534	37,152,367	7,168	9,753	324.56	267.59	98.32	1.00	0.68
Ref.	39	26,426,104	33,738,561	NA	NA	NA	NA	98.14	0.93	0.93

590 **Table 5. *Canu* and *Quickmerge* parameter optimization: contiguity and accuracy.** Assemblies refer to parameter sets defined in Table 3.

Species	Assembly	# Fragments	N50 (bp)	Largest fragment size (bp)	# global misassemblies	# local misassemblies	# Mismatches per 100 Kb	# Indels per 100 Kb	BUSCO score		
									complete genes	fragmented genes	missing genes
Dmel	P4	266	21,413,354	25,775,485	623	267	8.66	6.49	98.75	0.57	0.68
	P4f	267	21,413,185	25,776,014	919	318	10.55	6.73	98.75	0.57	0.68
	P4o	265	21,413,352	25,775,437	635	275	8.57	6.42	98.75	0.57	0.68
	P4o''	210	21,413,344	25,775,300	638	295	8.10	6.02	98.79	0.57	0.64
	P4fo''	211	21,413,185	25,776,408	943	340	9.90	6.20	98.79	0.57	0.64
	P4q	116	21,413,360	25,775,323	1,561	521	8.72	5.77	98.79	0.57	0.64
	P4r	113	21,450,483	25,801,485	1,751	582	8.66	5.75	98.61	0.75	0.64
	P4s	338	14,528,003	25,770,616	3,684	1,145	12.34	7.34	98.79	0.57	0.64
	Ref.	7	25,286,936	32,079,331	NA	NA	NA	NA	98.68	0.75	0.57
SON	P6	104	27,074,084	33,145,389	7,168	9,753	324.69	264.16	98.39	0.93	0.68
	P6f	103	27,125,966	33,144,936	7,173	9,760	324.80	264.49	98.36	0.96	0.68
	P6n	88	27,074,290	33,145,104	7,357	9,788	324.44	263.58	98.39	0.93	0.68
	P6n''	67	27,074,601	33,145,127	7,261	9,728	325.29	263.80	98.29	0.96	0.75
	P6fn''	66	27,125,795	33,144,975	7,271	9,727	325.23	263.97	98.25	1.00	0.75
	P6o	65	27,074,498	33,145,149	7,261	9,718	325.17	263.76	98.32	0.93	0.75
	P6q	62	27,074,467	33,145,189	7,470	9,752	325.83	263.63	98.32	0.93	0.75
	P6r	58	27,027,841	34,117,449	7,245	9,165	329.97	262.71	93.25	0.93	5.82
	P6s	151	27,122,727	34,181,614	9,159	10,205	328.59	263.06	98.14	0.93	0.93
	Ref.	39	26,426,104	33,738,561	NA	NA	NA	NA	98.14	0.93	0.9
CAT	P6n''	79	12,454,906	23,097,599	2,272	3,104	56.41	67.39	98.39	1.00	0.61
	P6fn''	79	12,457,238	23,102,169	2,274	2,949	56.40	65.70	98.64	0.82	0.54
	Ref.	39	26,866,924	34,148,556	NA	NA	NA	NA	98.11	0.93	0.96
	Ref. Miller et al. (2018)	122	5.0 Mb	NA	NA	NA	0.22	0.052	98	NA	NA

592 **Table 6. Improvement of contiguity and quality throughout the pipeline.** Abbreviations: Sr: short-read assembly; H: hybrid assembly; Lr:
593 long-read assembly; M: merged assembly; Q: Quiver polishing; P: Pilon polishing. Here MQP corresponds to P4 for Dmel and P6 for SON
594 (Tables 4, 5).

Species	Assembly	# Fragments	N50 (bp)	Largest fragment (bp)	# global misassemblies	# local misassemblies	# Mismatches per 100 kb	# Indels per 100 kb	BUSCO score		
									complete genes	fragmented genes	missing genes
Dmel	Sr	15,404	22,245	250,600	138	22	3.05	0.68	96.96	2.07	0.96
	H	302	5,369,803	20,351,387	449	391	28.34	570.44	66.02	15.51	18.47
	HQ	302	5,378,161	20,387,636	685	439	8.26	13.51	95.03	2.00	2.97
	HQP	302	5,378,529	20,385,575	689	464	8.91	7.78	97.89	0.57	1.54
	Lr	426	10,086,116	24,845,957	3,935	2,126	16.25	12.16	89.03	6.50	4.47
	LrQP	426	10,090,934	24,862,005	4,002	2,131	12.23	8.07	98.21	0.57	1.21
	M	266	21,413,390	25,776,101	603	288	8.95	7.19	98.75	0.57	0.68
	MQP	266	21,413,354	25,775,485	623	267	8.66	6.49	98.75	0.57	0.68
	Ref.	7	25,286,936	32,079,331	NA	NA	NA	NA	98.68	0.75	0.57
SON	Sr	57,046	3,385	45,376	346	2,539	219.24	200.08	89.10	7.93	2.97
	H	136	9,893,295	18,894,064	6,773	9,558	331.40	457.22	88.14	8.40	3.47
	HQ	136	9,840,048	18,808,757	6,898	9,646	324.72	271.29	98.54	0.93	0.54
	HQP	136	9,834,752	18,808,038	6,893	9,663	324.81	265.21	98.68	0.82	0.50
	Lr	343	2,678,315	8,942,850	8,790	9,912	332.66	316.57	96.57	1.93	1.50
	LrQP	343	2,679,816	8,945,441	9,055	10,035	328.02	262.04	98.14	1.04	0.82
	M	104	27,077,180	33,146,112	7,167	9,792	325.14	264.44	98.43	0.89	0.68
	MQP	104	27,074,084	33,145,389	7,168	9,753	324.69	264.16	98.39	0.93	0.68
	Ref.	39	26,426,104	33,738,561	NA	NA	NA	NA	98.14	0.93	0.9

595

Figures

Fig. 1. Size profile of DNA from *D. mojavensis* (Sonora) extracted with three different methods. Images of three gels, corresponding to each method, have been collated here, using the same ladder (sizes shown on the left).

607

608 **Fig. 2. DBG2OLC Pipeline, including the final merging step and the polishing steps.**

Fig. 3. Contiguity (A, D), error level (B, E) and Busco score (C, F) for Dmel (A-C) and SON (D-F) assemblies, at each step of the pipeline. Significantly larger values are printed above dashed lines. Assembly parameters are described in Table 6.

614

615 **Fig. 4. Alignment of SON merged assembly P6fn” (y-axis) on the *D. mojavensis* (Catalina) reference genome (x-axis).** Only fragments
 616 longer than 900 Kb are shown. Müller elements (chromosomes) of the reference genome (Catalina) are shown. Yellow boxes represent a
 617 single Müller element. Gray horizontal lines indicate the contigs from the SON assembly.

Appendix. Command lines to call each program used in the study.

(1) Data manipulation

Merge .bam files from several PacBio sequencing cells (node=1:ncpus=1:mem=6gb):
`~/Programs/SMRTtools/smrctools/bin/bamtools merge -list ListPBreadsFiles.fofn -out PBreads_all.bam`

Merge two .fasta files: (node=1:ncpus=1:mem=6gb):
`cat reads1.fasta reads2.fasta > reads1_and_2.fasta`

Convert .bax.h5 to .bam (node=1:ncpus=1:mem=6gb):
`~/Programs/SMRTtools/smrctools/bin/bax2bam my_movie.*.bax.h5 -o my_movie`

Convert .bam to .fastq (node=1:ncpus=1:mem=6gb):
`~/Programs/SMRTtools/smrctools/bin/samtools fastq my_reads.bam > my_reads.fastq`

OR
`~/Programs/SMRTtools/smrctools/bin/bam2fastq my_reads.bam -o my_reads`

Convert .bam to .fasta (node=1:ncpus=1:mem=6gb):
`~/Programs/SMRTtools/smrctools/bin/bam2fasta my_reads.bam -o my_reads`

Convert .fastq to .fasta with the *prinseq-lite* tool (v0.20.4) (node=1:ncpus=1:mem=6gb):
`perl prinseq-lite.pl -fastq my_reads.fastq -out_format 1`

Convert .fastq to .fasta with the *FASTX* toolkit (v0.0.13) (node=1:ncpus=1:mem=6gb):
`fastq_to_fasta -i my_reads.fastq -o my_reads.fasta`

(2) Short-read assembly

Trimming (with *Platanus* trimmer)
(node=1:ncpus=5:mem=30gb;cput=10:00:00;walltime=02:00:00):
`platanus_trim my_PE_reads_1.fastq my_PE_data_2.fastq -t 5`

Platanus assembler (node=1:ncpus=14:mem=65gb; cput=28:00:00;walltime=02:00:00):
`platanus assemble -o Shortread_contigs -f my_PE_reads_[12].fastq.trimmed -t 16 -m 75 2> Plat_SON2.log`

SparseAssembler, with kmer size 53 (node=1:ncpus=1:mem=6gb; cput=02:00:00; walltime=02:00:00):
`SparseAssembler LD 0 k 53 g 15 NodeCovTh 1 EdgeCovTh 0 GS 165000000 p1 my_PE_reads_1.fastq.trimmed p2 my_PE_reads_2.fastq.trimmed`

(3) Hybrid assembly

DBG2OLC step (node=1:ncpus=1:mem=6gb; cput=02:50:00;walltime=02:50:00):
`DBG2OLC k 17 KmerCovTh 2 MinOverlap 150 AdaptiveTh 0.002 LD1 0 MinLen 200 Contigs Shortread_contigs.fasta RemoveChimera 1 f my_PBreads.fasta`

Note: *DBG2OLC* has memory limitations. If run on node=1:ncpus=2:mem=12gb, it will still use one chore only, but more memory as allowed, and run twice as fast.

656 Building list of contigs identifiers (node=1:ncpus=1:mem=6gb;
 657 cput=00:05:00;walltime=00:05:00) (requires python2):
 658 [split_reads_by_backbone.py -b backbone_raw.fasta -o ./Cons_backbones -r](#)
 659 [Shortread_contigs_and_PBreads.fasta -c DBG2OLC_Consensus_info.txt](#)
 660 *Sparc* step ((node=1:ncpus=28:mem=90gb; cput=84:00:00;walltime=03:00:00):
 661 [sh split_and_run_sparc_ncpus_new.sh ./Cons_backbones 2 28 > Sparclog_Part1.txt](#)
 662
 663 (4) Long-read only assembly with *Canu*
 664 [canu -p PB_Only_Assembly -d /path_to_curr_dir genomeSize=123m correctedErrorRate=0.039 -](#)
 665 [useGrid=true -maxThreads=16 -maxMemory=90 -gridEngineThreadsOption="-l](#)
 666 [select=1:ncpus=16:mem=100gb" -gridEngineMemoryOption="-l walltime=02:00:00" -gridOptions="-W](#)
 667 [group_list=my_group_ID -q standard" -pacbio-raw /path_to_Pbreads/Pbreads.fasta](#)
 668 Note: Because the *Canu* pipeline calls a master script, more parameters, normally passed to the
 669 PBS script have to be sent to the command line when calling *Canu*. The option [-nanopore-raw](#) was
 670 used when assembling Nanopore reads.
 671
 672 (5) Assembly merging
 673 Using the *Quickmerge* wrapper, and with hybrid assembly as donor and long-read only assembly
 674 as acceptor (node=1:ncpus=1:mem=6gb; cput=00:25:00;walltime=00:25:00; requires python/3):
 675 [merge_wrapper.py Hybrid_assembly.fasta longread_assembly.fasta -l 10000000 -lm 10000](#)
 676
 677 (6) Quiver polishing
 678 Align PB reads (in .bam format) to assembly with *Pbalign* (node=1:ncpus=28:mem=168gb;
 679 cput=224:00:00;walltime=08:00:00):
 680 [~/Programs/SMRTtools/smrctools/bin/pbalign --nproc 28 my_PBreads.bam my_assembly.fasta](#)
 681 [aligned_PBreads.bam](#)
 682 Index aligned PB reads (node=1:ncpus=1:mem=6gb; cput=00:10:00;walltime=00:10:00):
 683 [~/Programs/SMRTtools/smrctools/bin/pbindex aligned_PBreads.bam](#)
 684 Index assembly (node=1:ncpus=1:mem=6gb; cput=00:02:00;walltime=00:02:00):
 685 [~/Programs/SMRTtools/smrctools/bin/samtools faidx my_assembly.fasta](#)
 686 Run Quiver (node=1:ncpus=28:mem=168gb; cput=154:00:00;walltime=05:30:00):
 687 [~/Programs/SMRTtools/smrctools/bin/quiver -j 28 -r my_assembly.fasta -o my_assembly_polished.fasta](#)
 688 [aligned_PBreads.bam](#)
 689 Run *Arrow* (node=1:ncpus=28:mem=168gb; cput=154:00:00;walltime=05:30:00):
 690 [~/Programs/SMRTtools/smrctools/bin/arrow -j 28 -r my_assembly.fasta -o my_assembly_polished.fasta](#)
 691 [aligned_PBreads.bam](#)
 692
 693 (7) Pilon polishing
 694 Index the assembly file (node=1:ncpus=6:mem=6gb; cput=00:05:00;walltime=00:05:00):
 695 [bowtie2-build my_assembly.fasta my_assembly](#)

```

696 Align short-reads to assembly with Bowtie2 (node=1:ncpus=28:mem=168gb;
697 cput=42:00:00;walltime=01:30:00):
698 bowtie2 -x my_assembly -1 my_PE_reads_1.fastq.trimmed -2 my_PE_reads_2.fastq.trimmed -S
699 Aligned_reads.sam -p 28

700 Convert .sam to .bam (node=1:ncpus=1:mem=6gb; cput=00:10:00;walltime=00:10:00):
701 samtools view -bS Aligned_reads.sam > Aligned_reads.bam

702 Sort reads (node=1:ncpus=28:mem=168gb; cput=07:00:00;walltime=00:15:00):
703 samtools sort Aligned_reads.bam -o Aligned_reads_sorted.bam -@ 28

704 Index reads (node=1:ncpus=6:mem=6gb; cput=00:02:00;walltime=00:02:00):
705 samtools index Aligned_reads_sorted.bam -@ 28

706 Run Pilon with paired ends only (node=1:ncpus=28:mem=168gb;
707 cput=14:00:00;walltime=00:30:00; requires java/8):
708 java -Xmx64G -jar pilon-1.22.jar --genome my_assembly.fasta --frags Aligned_PEREads_sorted.bam --
709 output ./my_assembly_polished --threads 28

710 Run Pilon with paired ends and mate pairs
711 (node=1:ncpus=28:mem=168gb; cput=28:00:00;walltime=01:00:00; requires java/8):
712 java -Xmx64G -jar pilon-1.22.jar --genome my_assembly.fasta --frags Aligned_PEREads_sorted.bam --
713 jumps Aligned_MPreads_sorted.bam --output ./my_assembly_polished --threads 14

714 Note: Pilon seems to try to use more cpus than allowed with the --thread option and requires more
715 memory to run on both paired ends and mate pairs. To counter this problem, we actually ran it on
716 28 cpus but passed less cpus (just enough to avoid memory issue) to the --thread option.

717

718 (8) Nanopolish polishing

719 Index the raw Nanopore reads (node=1:ncpus=1:mem=6gb; cput=02:30:00;walltime=02:30:00):
720 nanopolish index -d ./path_to_Fast5 -f List_summary.fofn -v ./Reads_basecalled_pass.fastq
721
722 Index the assembly file (node=1:ncpus=6:mem=6gb; cput=00:10:00;walltime=00:10:00):
723 bwa index my_assembly.fasta

724 Align short-reads to assembly with Bwa (node=1:ncpus=28:mem=168gb;
725 cput=161:00:00;walltime=05:45:00):
726 bwa mem -x ont2d -t 28 my_assembly.fasta /path_to_raw_reads/Reads_basecalled_pass.fastq | samtools
727 sort -o Reads.sorted.bam -@ 28
728 samtools index Reads.sorted.bam -@ 28

729 Run Nanopolish (node=1:ncpus=28:mem=168gb; cput=854:00:00;walltime=30:30:00):
730 python nanopolish_makerange.py my_assembly.fasta | cat > Chunks.txt

731 more Chunks.txt | parallel --results ./nanopol.res -P 7 nanopolish variants --consensus --faster -o
732 polished.{1}.vcf -w {1} -r ./Reads_basecalled_pass.fastq -b ./Reads.sorted.bam -g ./my_assembly.fasta -t
733 4 --min-candidate-frequency 0.2
734 Note: Nanopolish was unable to handle paths to directory others than the working directory,
735 therefore we placed both raw indexed reads, the draft assembly and the sorted reads in the same
736 directory.

737 Convert .vcf to .fasta (node=1:ncpus=1:mem=6gb; cput=00:03:00;walltime=00:30:00):

```


738 `nanopolish vcf2fasta -g ./my_assembly.fasta ./polished.*.vcf > ./my_assembly_polished.fasta`

739

740 (9) Quality assessment

741 *Quast* (node=1:ncpus=5:mem=30gb; cput=05:00:00;walltime=01:00:00;requires python/3)

742 `~/Programs/quast-4.6.2/quast.py -o ./ -R /path_to_reference_genome/Reference.fasta --threads 5 --min-`

743 `alignment 400 --no-plot --no-html --no-icarus --labels My_assembly --eukaryote`

744 `/path_to_assembly/My_assembly.fasta`

745 Note: walltime will depend on how divergent is the draft assembly from the reference assembly

746 *Busco* (node=1:ncpus=14:mem=84gb; cput=25:40:00;walltime=01:50:00; requires python/3,

747 *augustus*/3 and *hmmer*/3)

748 `python ~/Programs/busco/scripts/run_BUSCO.py -i /path_to_assembly/My_assembly.fasta -o`

749 `output_directory -l ~/Programs/busco/diptera_odb9/ -m genome -c 14 -sp fly --blast_single_core`

750

751 (10) Visualization with *Dot*

752 (node=1:ncpus=1:mem=6gb; cput=00:08:00;walltime=00:08:00)

753 `nucmer -c 100 -t 3 -prefix=my_assembly /path_to_reference/Reference_genome.fasta`

754 `/path_to_assembly/My_assembly_100KB.fasta`

755 `python DotPrep.py --delta my_assembly.delta --out outputname --unique-length 10000 --overview 500000`

756