bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Submission Type: Letter (Methods)

in silico Whole Genome Sequencer & Analyzer (iWGS): a computational pipeline to guide the design and analysis of *de novo* genome sequencing studies

Xiaofan Zhou[1],
David Peris[2],
Chris Todd Hittinger[2], and
Antonis Rokas[1,*]

[1]Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235, USA
[2]Laboratory of Genetics, Genome Center of Wisconsin, DOE Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, J. F. Crow Institute for the Study of Evolution, University of Wisconsin-Madison, Madison, WI 53706, USA

*Correspondence:
Antonis Rokas
Tel: +1-615-936-3892
Fax: +1-615-343-6707
E-mail: antonis.rokas@vanderbilt.edu

Key words: genome sequencing, high-throughput sequencing, *de novo* assembly, experimental design, simulation, non-model organism

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Abstract

The availability of genomes across the tree of life is highly biased toward vertebrates, pathogens, human disease models, and organisms with small and streamlined genomes. Recent progress in genomics has enabled the *de novo* decoding of the genome of virtually any organism, greatly expanding its potential for understanding the biology and evolution of the full spectrum of biodiversity. The increasing diversity of sequencing technologies, assays, and *de novo* assembly algorithms have augmented the complexity of *de novo* genome sequencing projects in non-model organisms. To reduce the costs and challenges in *de novo* genome sequencing projects and streamline their experimental design and analysis, we developed iWGS (*in silico* Whole Genome Sequencer and Analyzer), an automated pipeline for guiding the choice of appropriate sequencing strategy and assembly protocols. iWGS seamlessly integrates the four key steps of a *de novo* genome sequencing project: data generation (through simulation), data quality control, *de novo* assembly, and assembly evaluation and validation. The last three steps can also be applied to the analysis of real data. iWGS is designed to enable the user to have great flexibility in testing the range of experimental designs available for genome sequencing projects, and supports all major sequencing technologies and popular assembly tools. Three case studies illustrate how iWGS can guide the design of *de novo* genome sequencing projects and evaluate the performance of a wide variety of user-specified sequencing strategies and assembly protocols on genomes of differing architectures. iWGS, along with a detailed documentation, is freely available at http://as.vanderbilt.edu/rokaslab/tools.html.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Whole genome sequences are rich sources of information about organisms that are superbly useful for addressing a wide variety of evolutionary questions, such as measuring mutation rates (Kumar and Subramanian 2002), characterizing the genomic basis of adaptation (Roux, et al. 2014), and building the tree of life (Rokas, et al. 2003; Salichos and Rokas 2013). Until now, however, organismal diversity has been highly unevenly covered, and most sequenced genomes correspond to model organisms, organisms of medical or economic importance, or ones that have small and streamlined genomes (Reddy, et al. 2015).

The rapid advance of DNA sequencing technologies has dramatically reduced the labor and cost required for genome sequencing, which is evidenced by the burst of large-scale genome projects in recent years that includes, for example, the 1,000 Fungal Genomes (1KFG) Project (Grigoriev, et al. 2011), the Insect 5K Project (Robinson, et al. 2011), and the Genome 10K Project (Genome 2009). Some of these projects have already begun to fuel important discoveries in evolution and other fields (Zhang, et al. 2014). Equally importantly, high-throughput DNA sequencing has made it possible for single investigators to perform *de novo* genome sequencing in virtually any organism they are interested in (Rokas and Abbot 2009). Such sequencing efforts may target various organisms with a large diversity of genome architectures. Therefore, to achieve optimal results, the choice of sequencing strategy (i.e. the combination of sequencing technology (e.g. Illumina, Pacific Biosciences), sequencing assay (e.g. paired-end, mate-pair), and other variables, such as sequencing depth) and assembly protocols (e.g. assemblers and the associated parameters) should ideally be tailored to the characteristics of a given genome, such as size and GC/repeat content (Nagarajan and Pop 2013).

The vast majority of *de novo* sequenced genomes have been generated using the Illumina technology, either solely or in combination with other technologies (Reddy, et al. 2015). This is largely due to the Illumina technology's ability to quickly generate tens to hundreds of millions of highly accurate short sequence reads of up to 300 bases per run at very low per base cost (Glenn 2011). Additionally, the Illumina technology offers two powerful sequencing assays, paired-end (PE) and mate-pair (MP), which generate sequence read pairs that span short (hundreds of base-pairs) and relatively long

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

(thousands of base-pairs) genomic regions, respectively. Mixing multiple PE and MP libraries with different insert sizes allows for highly flexible sequencing strategies, and several state-of-the-art assembly algorithms have been developed that exploit all these advantages. For instance, the *de novo* genome assembler ALLPATHS-LG can generate finished or near-finished assemblies for small genomes (e.g. microbial) using only Illumina short-read data by including both MP and overlapping PE libraries (Ribeiro, et al. 2012). On its own, however, the Illumina technology performs less well for larger and less streamlined genomes, mainly due to the short lengths of Illumina sequence reads and the technology's bias against certain genomic regions (e.g. GC-rich) (Ross, et al. 2013).

The Pacific Biosciences (PacBio) technology generates sequence reads that are substantially longer and have much less sequencing bias, albeit at the cost of a substantially lower per-read accuracy; the average read length increases to above 10 kilo-bases with the latest chemistry but displays only ~87% accuracy (Koren and Phillippy 2015). Thus, this technology is particularly useful for the sequencing of complex genomes (Koren and Phillippy 2015). Recent developments in both sequencing chemistry and assembly algorithms have enabled PacBio-only *de novo* assembly for microbial genomes (Koren, et al. 2013), but the high sequence coverage required for this approach remains cost-prohibitive for large eukaryotic genomes. Nevertheless, in combination with more affordable Illumina short-read data, PacBio long reads – even at low coverage – can lead to significantly improved assemblies (Utturkar, et al. 2014).

De novo genome sequencing projects are further complicated by the large array of assembly software tools, which differ in many aspects, such as algorithmic design, supported/required data types, and computational efficiency (Nagarajan and Pop 2013; Simpson and Pop 2015). Systematic evaluations of assembly programs show that no single assembler is the best across all circumstances; rather, an assembler's performance critically depends on genome complexity and sequencing strategy adopted (Earl, et al. 2011; Bradnam, et al. 2013). Moreover, many assemblers use adjustable parameters (e.g. the k-mer size for *de Bruijn* assemblers), the values of which can critically affect the assembly quality. In practice, such parameters are often selected intuitively or through the time-consuming process of testing multiple values (Chikhi and Medvedev 2014).

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

The great number of possible ways to combine sequencing technologies, assays, and assembly algorithms poses a great challenge for the experimental design and data analysis in *de novo* genome sequencing projects, which in turn can sometimes lead to poor quality or downright incorrect assemblies (Denton, et al. 2014). As a consequence, several pipelines have been developed to automate specific steps in the process; for example, the recently developed iMetAMOS (Koren, et al. 2014) and RAMPART (Mapleson, et al. 2015) have been specifically designed to automate genome assembly. However, as *de novo* genome sequencing is increasingly adopted by single investigator laboratories, there is an urgent need for streamlined approaches that enable investigators to not only efficiently generate high-quality draft genome assemblies but also to predict (via simulation) and identify the most suitable design(s) (i.e. the most suitable combination(s) of sequencing strategy and assembly protocol) currently available for a specific genome.

To address this need, we have developed an automated pipeline for the design and execution of *de novo* genome sequencing projects that we name iWGS (*in silico* Whole Genome Sequencer and Analyzer). To approximate the performance of different sequencing strategies and assembly protocols, iWGS simulates high-throughput genome sequencing on user-provided reference genomes (e.g. genomes that closely represent the characteristics of the real targets), facilitating the identification of optimal experimental designs. iWGS allows users to experiment with various combinations of sequencing technologies, assays, assembly tools, and relevant parameters in a single run. iWGS is also designed to work with real data and can be used as a convenient tool for automated selection of the best assembly or genome assembler. Finally, using three case studies, each one focused on specific challenges frequently encountered in *de novo* genome sequencing studies (e.g. high repeat content, biased nucleotide composition, etc.), we illustrate how iWGS can be applied to guiding the design and analysis of *de novo* genome sequencing studies.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

New Approaches: iWGS

iWGS encompasses all major steps of a typical *de novo* genome sequencing study, including the generation of sequence reads, data quality control, *de novo* assembly, and evaluation of assemblies (Fig. 1).

1) **Simulation:** iWGS uses realistic high-throughput sequencing (HTS) read simulators ART (Huang, et al. 2012), pIRS (Hu, et al. 2012), and PBSIM (Ono, et al. 2013) to generate Illumina and PacBio sequence reads from a given user-specified genome. These programs can simulate all popular data types, including Illumina PE and MP sequence reads, as well as PacBio continuous long sequence reads. Furthermore, they mimic sequencing errors and nucleotide composition biases in real data by using empirical profiles of these artefacts, which can be easily customized to stay current with upgrades in sequencing technologies. For instance, we have created a quality-score frequency profile learned from sequence reads generated by latest PacBio chemistry to better reflect the improved sequence read accuracy. This simulation step can be omitted when the goal is the analysis of real data.

2) **Quality control:** HTS data generated by all technologies contain errors and artefacts, which may sometimes substantially compromise the quality of the assembly (Zhou and Rokas 2014). Therefore, iWGS includes an optional step to perform pre-processing of the data, including trimming of low-quality bases, removal of adapter contaminations, and correction of sequencing errors. Since some assemblers [e.g. ALLPATHS-LG (Ribeiro, et al. 2012)] have their own pre-processing modules, iWGS automatically determines for each assembly protocol whether to use the original or the processed data.

3) **Assembly:** To maximize users' flexibility in experimental design, iWGS supports 11 *de novo* genome assembly tools [ABYSS (Simpson, et al. 2009), ALLPATHS-LG (Ribeiro, et al. 2012), Celera Assembler (Miller, et al. 2008; Koren, et al. 2013), DISCOVAR (Weisenfeld, et al. 2014), MaSuRCA (Zimin, et al. 2013), Minia (Salikhov, et al. 2013), Platanus (Kajitani, et al. 2014), SGA (Simpson and Durbin 2012), SOAPdenovo2 (Luo, et al. 2012), SPAdes (Bankevich, et al. 2012), and Velvet

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

(Zerbino and Birney 2008)]; all but DISCOVAR, Minia, and Platanus participated in recent large-scale assembler comparisons (Bradnam, et al. 2013; Magoc, et al. 2013). These supported assemblers allow users to carry out *de novo* assembly using only Illumina short-read data (e.g. SOAPdenovo2) and only PacBio long-read data (e.g. Celera Assembler), or to perform hybrid assembly that uses both (e.g. SPAdes). To achieve the best possible results while avoiding the computationally expensive process of testing multiple combinations of parameters, iWGS takes advantage of successful assembly recipes (i.e., recommended settings for each assembler) established in studies such as Assemblathon 2 (Bradnam, et al. 2013) and GAGE-B (Magoc, et al. 2013), and uses KmerGenie to determine the optimal k-mer size (Chikhi and Medvedev 2014).

4) **Evaluation:** iWGS uses QUAST (Gurevich, et al. 2013) to evaluate all generated assemblies. In addition to providing basic statistics like N50 (the largest contig/scaffold size wherein half of the total assembly size is contained in contigs/scaffolds no shorter than this value), QUAST compares each assembly against the reference genome (in the case of simulations) and generates a number of highly informative quality matrices, such as mis-assemblies, assembled sequences not present in the reference (and vice versa), and genes recovered in the assembly if the reference genome is annotated. At the end, iWGS ranks all assemblies based on selected matrices in the QUAST report using a previously described weighting strategy (Abbas, et al. 2014). This ranking, along with the detailed QUAST report, helps users to identify the best overall assembly, as well as the corresponding combination of sequencing strategy and assembly protocol.

iWGS is designed with flexibility and ease-of-use in mind to allow users to readily examine various experimental designs; each data set may be used multiple times in different assembly protocols, and each assembler may be run repeatedly with different input data sets. Multiple sequencing strategies and assembly protocols can be specified straightforwardly in one configuration file; only a few parameters are required for each strategy/protocol, while other settings (e.g. quality profiles for read simulation) are globally shared across strategies/protocols of the same type. Alternatively, advanced

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

users can opt to customize the strategies/protocols so that, for example, each sequencing data set is simulated with different quality settings. Furthermore, iWGS rigorously checks the configurations for issues such as the compatibility between sequencing strategies and assembly protocols.

iWGS is a lightweight pipeline written in Perl with minimal dependencies. The source code, detailed documentation, and example test sets are freely available at http://as.vanderbilt.edu/rokaslab/tools.html. For the convenience of users, we also include in the package pre-complied binaries of supported third-party software tools, when available, all of which are redistributed under proper licenses.

Case Studies

To demonstrate the use of iWGS and provide examples of its utility, we developed three case studies where iWGS was used to guide the selection of sequencing strategy for genomes representing a wide range of sizes and complexity levels (Supplementary Table S1). The competing strategies were selected to enable both Illumina-only and PacBio-only assemblies, as well as hybrid assembly of the two data types (Table 1).

Case study I (Repetitive content issue). We first compared the sequencing of two fungi, *Zymoseptoria tritici* (synonym: *Mycosphaerella graminicola*) (Goodwin, et al. 2011) and *Pseudocercospora fijiensis* (synonym: *Mycosphaerella fijiensis*) (Ohm, et al. 2012), which both belong to the class Dothideomycetes yet have dramatically different repeat contents; the estimated repeat contents are ~15% and ~50% for the two genomes, respectively. Our simulations showed that, while good quality assemblies can be obtained for *Z. tritici* using either data type, the PacBio-only assembly for *Ps. fijiensis* vastly outperforms assemblies based on Illumina data alone (Fig. 2). The results are consistent with the notion that PacBio long reads are particularly powerful in resolving repeats (Koren, et al. 2013). We then further tested if these results are informative for guiding the sequencing of another highly repetitive Dothideomycetes genome, *Cenococcum*

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

geophilum, which has a repeat content of c.a. 76% (http://genome.jgi.doe.gov/Cenge3). For *C. geophilum*, the PacBio-only assembly was again found to be the best, while the Illumina-only assembly using ALLPATHS-LG was ranked second (Fig. 2). The results nicely recapitulate those of *Ps. fijiensis*, suggesting that the use of iWGS would provide critical information for to help end users choose a successful sequencing of highly repetitive genomes that share similar characteristics. Importantly, since simulated assemblies are recoverable, the likely impact of the different assembly strategies on genes, gene families, or pathways of interest could also be examined in detail.

Case Study II (GC content and mtDNA assembly issue). We next examined the *de novo* assembly of mitochondrial genomes from whole genome sequencing data of *Saccharomyces cerevisiae* (Mewes, et al. 1997; Foury, et al. 1998). Yeast mitochondrial genomes are valuable resources for evolutionary and functional studies (Freel, et al. 2015), yet the acquisition of finished mitochondrial genome assemblies is not trivial because of their very low GC-content (~17%). We simulated a genome sequencing experiment using the nuclear and mitochondrial genomes of *S. cerevisiae*. We tested two ratios of nuclear to mitochondrial genome copy numbers representing low (1:50) and high (1:200) mitochondrial contents respectively (Solieri 2010). iWGS analysis showed that the *S. cerevisiae* mitochondrial genome was fully recovered at both low and high mitochondrial contents using Illumina data (Table 2). Consistent with recent observations made during the assembly of the *Saccharomyces eubayanus* genome, only certain assemblers performed well; for example, ALLPATHS-LG performed surprisingly poorly, while SPAdes performed quite well (Baker, et al. 2015). Importantly, the complete mitochondrial genome in a single contig can be obtained using Illumina data alone, as well as when low-coverage PacBio data is included (Table 2). Similarly, both Illumina and PacBio data resulted in good quality assemblies of the nuclear genome (Supplementary Table S2).

Case Study III (Genomic architecture issue). Lastly, we applied iWGS to three model eukaryotic genomes from different kingdoms and with different genomic architectures. Specifically, we analyzed *Drosophila melanogaster* (Adams, et al. 2000) and *Arabidopsis thaliana* (Arabidopsis Genome 2000), which are medium-sized animal

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

and plant genomes, respectively, as well as *Plasmodium falciparum* (Gardner, et al. 2002), a smaller protist genome with extremely low GC-content (~19%). For both *D. melanogaster* and *A. thaliana*, the best assembly was generated by using only PacBio data (Table 3). However, the PacBio-only assembly of *Pl. falciparum* was ranked behind two Illumina-only assemblies (Table 3), suggesting that, while PacBio data would perform well in general, caution may be needed for genomes with extreme characteristics, such as very low GC content.

Discussion

The design and analysis of *de novo* genome sequencing experiments is not trivial. On the design front, one has to balance between the complexity of the target genome, the strengths and weaknesses of each sequencing technology, and, importantly, the cost. Analysis is also challenging, as one is faced with multiple different algorithms and dozens of parameters. Although substantial efforts have been made to benchmark different approaches for genome assembly (Earl, et al. 2011; Salzberg, et al. 2012; Bradnam, et al. 2013; Magoc, et al. 2013), much less attention has been paid to investigating start-to-finish optimal sequencing strategies for a given genome.

iWGS is an automated tool that allows users to explicitly compare alternative experimental designs by using simulated sequencing data, even allowing users to estimate costs when these are known for the generation of each data type. We have illustrated the utility of iWGS in several case studies on mitochondrial and nuclear genomes with varying levels of complexity. For instance, our simulations suggest that Illumina-only sequencing strategies may be economical choices for the sequencing of regular genomes (e.g. *Z. tritici*), whereas PacBio data would be highly desirable for genomes of greater complexity (e.g. *Ps. fijiensis* and *C. geophilum*).

One key function of iWGS is the use of simulation data generated from a related reference genome to inform the experimental design for organisms lacking genomic data. A similar concept was previously used to evaluate sequencing strategies by using the rice

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

genome as the reference (Haiminen, et al. 2011). However, the related reference genomes available do not always resemble the characteristics of the sequencing target. One solution is to start with a closely related reference genome and tune it toward the target (e.g. adjust GC- and repeat contents) by using third-party tools that simulate genome-wide evolution (Arenas and Posada 2014) before running iWGS. Alternatively, one may simply use iWGS with reference genomes that are of comparable complexity regardless of the evolutionary relatedness.

Other important features of iWGS include the support for both Illumina short and PacBio long sequence reads and, correspondingly, the software tools compatible with these data types, as well as the ability to analyze real data. Along these directions, several further developments can be envisioned. First, supports for additional sequencing technologies, such as Oxford Nanopore, can be added as they become commercially available. In fact, the Celera Assembler and SPAdes assemblers, which are supported by iWGS, can already utilize nanopore reads (Miller, et al. 2008; Bankevich, et al. 2012). Similarly, realistic simulation of nanopore data will be possible once the patterns of errors and biases are better characterized using real data. Second, iWGS currently uses QUAST to evaluate genome assemblies, which is much less powerful without a reference. Other tools that utilize sequence read data for assembly evaluation can be implemented to better suit real data analysis (Hunt, et al. 2013; Rahman and Pachter 2013). Lastly, as iWGS provides users the flexibility to run multiple assembly protocols, there is a great opportunity to achieve an even better final assembly by combining competing assemblies using recently developed meta-assembly approaches (Wences and Schatz 2015). In summary, iWGS is a flexible, expandable, and easy to use pipeline that will aid in the design and execution of genome assembly experiments across the tree of life.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Acknowledgements

We thank Francis Martin for providing access to unpublished genome data for the Cenococcum geophilum genome produced by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. We thank Branden Timm for technical support on the WEI cluster. This work was conducted in part using the resources of the Advanced Computing Center for Research and Education at Vanderbilt University and the Wisconsin Energy Institute (WEI) cluster at the University of Wisconsin-Madison. This work was funded by the National Science Foundation (DEB-1442113 to AR; DEB-1253634 and DEB-1442148 to CTH), in part by the DOE Great Lakes Bioenergy Research Center (DOE Office of Science BER DE-FC02- 07ER64494 to CTH), and the National Institutes of Health (NIAID AI105619 to AR). CTH is an Alfred Toepfer Faculty Fellow, supported by the Alexander von Humboldt Foundation. CTH is a Pew Scholar in the Biomedical Sciences, supported by the Pew Charitable Trusts.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

References

Abbas MM, Malluhi QM, Balakrishnan P. 2014. Assessment of de novo assemblers for draft genomes: a case study with fungal genomes. *BMC Genomics*. 15 Suppl 9:S10.

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, et al. 2000. The genome sequence of Drosophila melanogaster. *Science*. 287:2185-2195.

Arabidopsis Genome I. 2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature*. 408:796-815.

Arenas M, Posada D. 2014. Simulation of genome-wide evolution under heterogeneous substitution models and complex multispecies coalescent histories. *Mol Biol Evol*. 31:1295-1301.

Baker E, Wang B, Bellora N, Peris D, Hulfachor AB, Koshalek JA, Adams M, Libkind D, Hittinger CT. 2015. The Genome Sequence of Saccharomyces eubayanus and the Domestication of Lager-Brewing Yeasts. *Mol Biol Evol*. DOI: 10.1093/molbev/msv168.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*. 19:455-477.

Bradnam KR, Fass JN, Alexandrov A, Baranay P, Bechner M, Birol I, Boisvert S, Chapman JA, Chapuis G, Chikhi R, et al. 2013. Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species. *Gigascience*. 2:10.

Chikhi R, Medvedev P. 2014. Informed and automated k-mer size selection for genome assembly. *Bioinformatics*. 30:31-37.

Denton JF, Lugo-Martinez J, Tucker AE, Schrider DR, Warren WC, Hahn MW. 2014. Extensive error in the number of genes inferred from draft genome assemblies. *PLoS Comput Biol*. 10:e1003998.

Earl D, Bradnam K, St John J, Darling A, Lin D, Fass J, Yu HO, Buffalo V, Zerbino DR, Diekhans M, et al. 2011. Assemblathon 1: a competitive assessment of de novo short read assembly methods. *Genome Res*. 21:2224-2241.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Foury F, Roganti T, Lecrenier N, Purnelle B. 1998. The complete sequence of the mitochondrial genome of Saccharomyces cerevisiae. *FEBS Lett*. 440:325-331.

Freel KC, Friedrich A, Schacherer J. 2015. Mitochondrial genome evolution in yeasts: an all-encompassing view. *FEMS Yeast Res*. 15:fov023.

Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, et al. 2002. Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature*. 419:498-511.

Genome KCoS. 2009. Genome 10K: a proposal to obtain whole-genome sequence for 10,000 vertebrate species. *J Hered*. 100:659-674.

Glenn TC. 2011. Field guide to next-generation DNA sequencers. *Mol Ecol Resour*. 11:759-769.

Goodwin SB, M'Barek S B, Dhillon B, Wittenberg AH, Crane CF, Hane JK, Foster AJ, Van der Lee TA, Grimwood J, Aerts A, et al. 2011. Finished genome of the fungal wheat pathogen Mycosphaerella graminicola reveals dispensome structure, chromosome plasticity, and stealth pathogenesis. *PLoS Genet*. 7:e1002070.

Grigoriev IV, Cullen D, Goodwin SB, Hibbett D, Jeffries TW, Kubicek CP, Kuske C, Magnuson JK, Martin F, Spatafora JW, et al. 2011. Fueling the future with fungal genomics. *Mycology*. 2:192-209.

Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 29:1072-1075.

Haiminen N, Kuhn DN, Parida L, Rigoutsos I. 2011. Evaluation of methods for de novo genome assembly from high-throughput sequencing reads reveals dependencies that affect the quality of the results. *PLoS One*. 6:e24182.

Hu X, Yuan J, Shi Y, Lu J, Liu B, Li Z, Chen Y, Mu D, Zhang H, Li N, et al. 2012. pIRS: Profile-based Illumina pair-end reads simulator. *Bioinformatics*. 28:1533-1535.

Huang W, Li L, Myers JR, Marth GT. 2012. ART: a next-generation sequencing read simulator. *Bioinformatics*. 28:593-594.

Hunt M, Kikuchi T, Sanders M, Newbold C, Berriman M, Otto TD. 2013. REAPR: a universal tool for genome assembly evaluation. *Genome Biol*. 14:R47.

Kajitani R, Toshimoto K, Noguchi H, Toyoda A, Ogura Y, Okuno M, Yabana M, Harada M, Nagayasu E, Maruyama H, et al. 2014. Efficient de novo assembly of highly

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

heterozygous genomes from whole-genome shotgun short reads. *Genome Res*. 24:1384-1395.

Koren S, Harhay GP, Smith TP, Bono JL, Harhay DM, McVey SD, Radune D, Bergman NH, Phillippy AM. 2013. Reducing assembly complexity of microbial genomes with single-molecule sequencing. *Genome Biol*. 14:R101.

Koren S, Phillippy AM. 2015. One chromosome, one contig: complete microbial genomes from long-read sequencing and assembly. *Curr Opin Microbiol*. 23:110-120.

Koren S, Treangen TJ, Hill CM, Pop M, Phillippy AM. 2014. Automated ensemble assembly and validation of microbial genomes. *BMC Bioinformatics*. 15:126.

Kumar S, Subramanian S. 2002. Mutation rates in mammalian genomes. *Proc Natl Acad Sci U S A*. 99:803-808.

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, et al. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience*. 1:18.

Magoc T, Pabinger S, Canzar S, Liu X, Su Q, Puiu D, Tallon LJ, Salzberg SL. 2013. GAGE-B: an evaluation of genome assemblers for bacterial organisms. *Bioinformatics*. 29:1718-1725.

Mapleson D, Drou N, Swarbreck D. 2015. RAMPART: a workflow management system for de novo genome assembly. *Bioinformatics*. 31:1824-1826.

Mewes HW, Albermann K, Bahr M, Frishman D, Gleissner A, Hani J, Heumann K, Kleine K, Maierl A, Oliver SG, et al. 1997. Overview of the yeast genome. *Nature*. 387:7-65.

Miller JR, Delcher AL, Koren S, Venter E, Walenz BP, Brownley A, Johnson J, Li K, Mobarry C, Sutton G. 2008. Aggressive assembly of pyrosequencing reads with mates. *Bioinformatics*. 24:2818-2824.

Nagarajan N, Pop M. 2013. Sequence assembly demystified. *Nat Rev Genet*. 14:157-167.

Ohm RA, Feau N, Henrissat B, Schoch CL, Horwitz BA, Barry KW, Condon BJ, Copeland AC, Dhillon B, Glaser F, et al. 2012. Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. *PLoS Pathog*. 8:e1003037.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Ono Y, Asai K, Hamada M. 2013. PBSIM: PacBio reads simulator--toward accurate genome assembly. *Bioinformatics*. 29:119-121.

Rahman A, Pachter L. 2013. CGAL: computing genome assembly likelihoods. *Genome Biol*. 14:R8.

Reddy TB, Thomas AD, Stamatis D, Bertsch J, Isbandi M, Jansson J, Mallajosyula J, Pagani I, Lobos EA, Kyrpides NC. 2015. The Genomes OnLine Database (GOLD) v.5: a metadata management system based on a four level (meta)genome project classification. *Nucleic Acids Res*. 43:D1099-1106.

Ribeiro FJ, Przybylski D, Yin S, Sharpe T, Gnerre S, Abouelleil A, Berlin AM, Montmayeur A, Shea TP, Walker BJ, et al. 2012. Finished bacterial genomes from shotgun sequence data. *Genome Res*. 22:2270-2277.

Robinson GE, Hackett KJ, Purcell-Miramontes M, Brown SJ, Evans JD, Goldsmith MR, Lawson D, Okamuro J, Robertson HM, Schneider DJ. 2011. Creating a buzz about insect genomes. *Science*. 331:1386.

Rokas A, Abbot P. 2009. Harnessing genomics for evolutionary insights. *Trends Ecol Evol*. 24:192-200.

Rokas A, Williams BL, King N, Carroll SB. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature*. 425:798-804.

Ross MG, Russ C, Costello M, Hollinger A, Lennon NJ, Hegarty R, Nusbaum C, Jaffe DB. 2013. Characterizing and measuring bias in sequence data. *Genome Biol*. 14:R51.

Roux J, Privman E, Moretti S, Daub JT, Robinson-Rechavi M, Keller L. 2014. Patterns of positive selection in seven ant genomes. *Mol Biol Evol*. 31:1661-1685.

Salichos L, Rokas A. 2013. Inferring ancient divergences requires genes with strong phylogenetic signals. *Nature*. 497:327-331.

Salikhov K, Sacomoto G, Kucherov G. 2013. Using Cascading Bloom Filters to Improve the Memory Usage for de Brujin Graphs. In: Darling A, Stoye J, editors. Algorithms in Bioinformatics: Springer Berlin Heidelberg. p. 364-376.

Salzberg SL, Phillippy AM, Zimin A, Puiu D, Magoc T, Koren S, Treangen TJ, Schatz MC, Delcher AL, Roberts M, et al. 2012. GAGE: A critical evaluation of genome assemblies and assembly algorithms. *Genome Res*. 22:557-567.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Simpson JT, Durbin R. 2012. Efficient de novo assembly of large genomes using compressed data structures. *Genome Res*. 22:549-556.

Simpson JT, Pop M. 2015. The Theory and Practice of Genome Sequence Assembly. *Annu Rev Genomics Hum Genet*. 16:153-172.

Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. *Genome Res*. 19:1117-1123.

Solieri L. 2010. Mitochondrial inheritance in budding yeasts: towards an integrated understanding. *Trends Microbiol*. 18:521-530.

Utturkar SM, Klingeman DM, Land ML, Schadt CW, Doktycz MJ, Pelletier DA, Brown SD. 2014. Evaluation and validation of de novo and hybrid assembly techniques to derive high-quality genome sequences. *Bioinformatics*. 30:2709-2716.

Weisenfeld NI, Yin S, Sharpe T, Lau B, Hegarty R, Holmes L, Sogoloff B, Tabbaa D, Williams L, Russ C, et al. 2014. Comprehensive variation discovery in single human genomes. *Nat Genet*. 46:1350-1355.

Wences A, Schatz M. 2015. Metassembler: merging and optimizing de novo genome assemblies. *Genome Biol*. 16:207.

Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res*. 18:821-829.

Zhang G, Li C, Li Q, Li B, Larkin DM, Lee C, Storz JF, Antunes A, Greenwold MJ, Meredith RW, et al. 2014. Comparative genomics reveals insights into avian genome evolution and adaptation. *Science*. 346:1311-1320.

Zhou X, Rokas A. 2014. Prevention, diagnosis and treatment of high-throughput sequencing data pathologies. *Mol Ecol*. 23:1679-1700.

Zimin AV, Marcais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. *Bioinformatics*. 29:2669-2677.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Table 1. Sequencing strategies and assembly protocols evaluated in the three case studies.

	Name	Read type[a]	Parameters
Sequencing strategies	LIB1	Illumina PE	depth: 50x; read length: 100bp; insert size: 180bp ± 9bp
	LIB2	Illumina MP	depth: 50x; read length: 100bp; insert size: 8000bp ± 400bp
	LIB3	Illumina PE	depth: 50x; read length: 250bp; insert size: 450bp ± 23bp
	LIB4	PacBio CLR	depth: 60x; read accuracy: 0.87 ± 0.03; read length: 11500bp ± 8000bp
	LIB5	PacBio CLR	depth: 10x; read accuracy: 0.87 ± 0.03; read length: 11500bp ± 8000bp
	Name	Assembler	Sequencing strategies used for assembly
Assembly protocols	ILMN1	ABYSS	LIB1, LIB2 (Illumina-only)
	ILMN2	ALLPATHS-LG	
	ILMN3	MaSuRCA	
	ILMN4	SGA	
	ILMN5	SOAPdenov2	
	ILMN6	SPAdes	
	ILMN7	Velvet	
	ILMN8	DISCOVAR	LIB3 (Illumina-only)
	PACB	Celera Assembler	LIB4 (PacBio-only)
	HYBR	SPAdes	LIB1, LIB2, LIB5 (Hybrid)

[a]Read type: PE – Paired-end; MP – Mate pair; CLR: Continuous long read.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Table 2. Performance of all experimental design evaluated in case study II.

Nuclear:mito genome ratio	Strategies			
	Complete, single contig assembly	Coverage ≥ 99%	20% ≤ Coverage < 99%	Coverage < 20%
1:50 (low mitochondrial content)	ILMN1, ILMN6, ILMN8, HYBR	ILMN7	ILMN2, ILMN4, ILMN5, PACB	ILMN3
1:200 (high mitochondrial content)	HYBR	ILMN6, ILMN7	ILMN1, ILMN8, PACB	ILMN2, ILMN3, ILMN4, ILMN5

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Table 3. Summary of top-ranking assemblies generated in case study III.

Organism (genome size)	Top 2 assemblies	Assembly statistics[1]		
		Scaffold N50 (kb)	Largest scaffold (kb)	Coverage
D. melanogaster (137.55 Mb)	PACB	443.8	3584.5	98.5%
	ILMN8	155.0	1007.7	91.8%
A. thaliana (119.15 Mb)	PACB	626.9	2603.0	99.8%
	ILMN8	266.6	2533.4	98.5%
Pf. falciparum (23.29 Mb)	ILMN8	222.0	729.9	98.4%
	ILMN2	28.0	146.2	96.7%

[1]All statistics shown are after correction using the reference genome.

bioRxiv preprint doi: https://doi.org/10.1101/028134; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Figure Legend

Figure 1. iWGS workflow. A typical iWGS analysis consists of four steps: 1) data simulation (optional); 2) preprocessing (optional); 3) *de novo* assembly; and 4) assembly evaluation. iWGS supports both Illumina short reads and PacBio long reads, and a wide selection of assemblers to enable *de novo* assembly using either or both types of data. Users can start the analysis simulating data drawn from a reference genome assembly or, alternatively, use real sequencing data as input and skip the simulation step.

Figure 2. Performance comparison of four representative experimental designs on three Dothideomycetes genomes. The four designs shown include two Illumina-only designs (ILMN2: ALLPATHS-LG and ILMN8: DISCOVAR), one PacBio-only design (PACB), and one hybrid design (HYBR: SPAdes). The statistics on coverage (recovered fraction of the reference genome), scaffold N50, and largest scaffold size are all after correction using the reference genome. Scaffold N50 and largest scaffold size are shown in log10 scale.

bioRxiv preprint doi: https://doi.org/10.1101/028124; this version posted November 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Figure 1

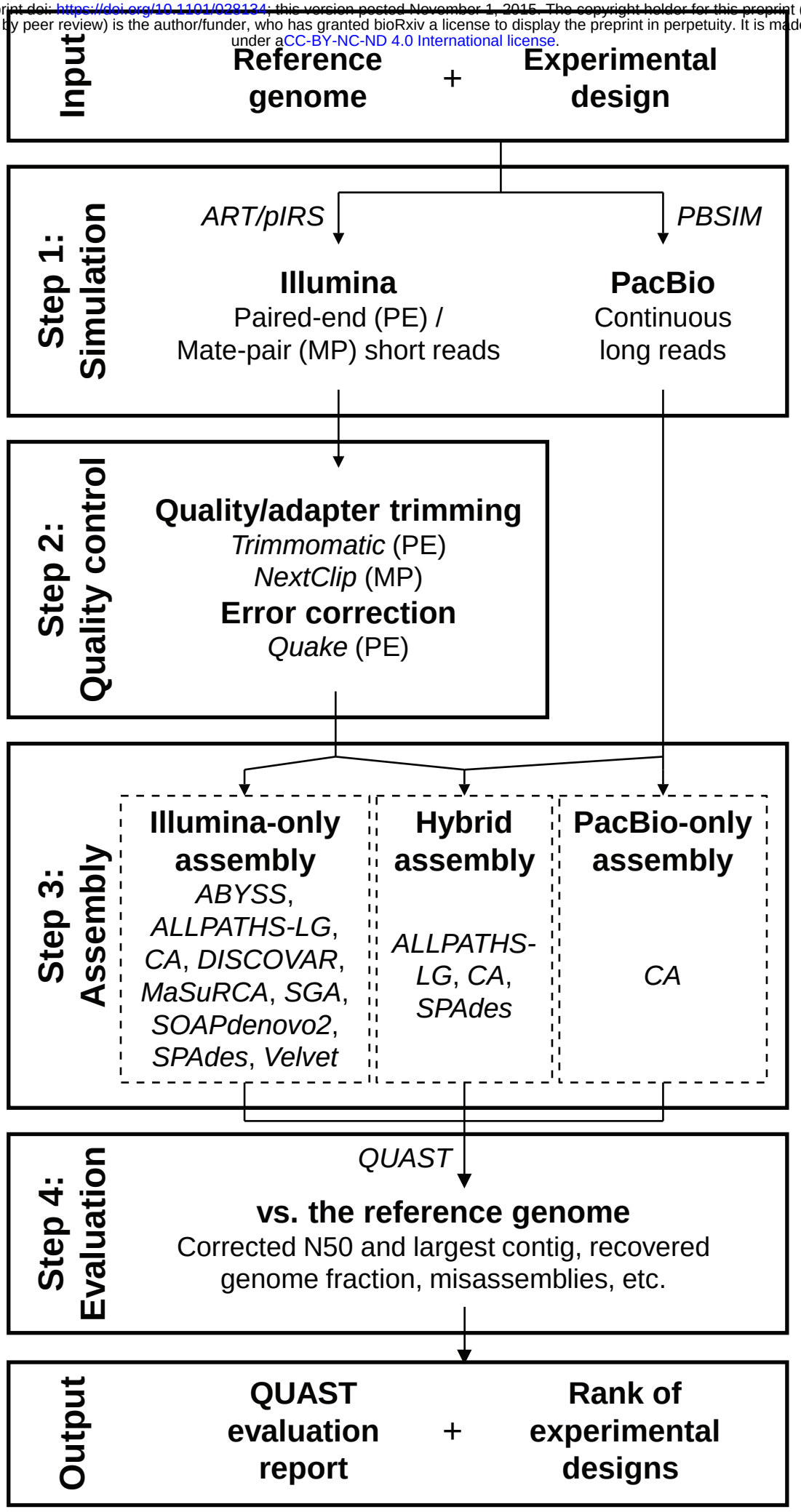
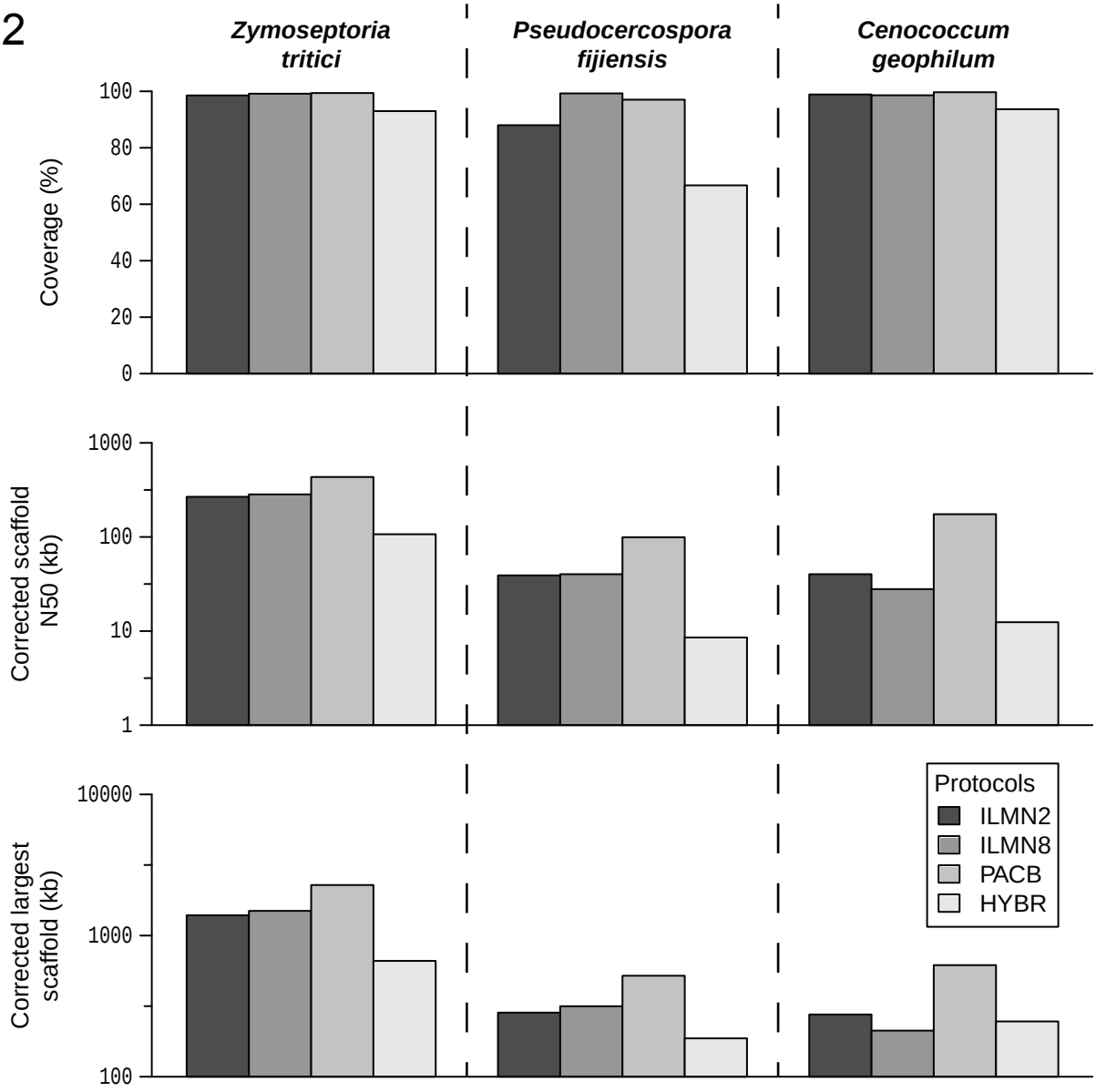


Figure 2


Zymoseptoria tritici
Pseudocercospora fijiensis
Cenococcum geophilum
Coverage (%)
100
80
60
40
20
0
Corrected scaffold N50 (kb)
1000
100
10
1
Corrected largest scaffold (kb)
10000
1000
100
Protocols
ILMN2
ILMN8
PACB
HYBR