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

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

(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
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](http://as.vanderbilt.edu/rokaslab/tools.html). For the convenience of users, we also include in the package pre-compiled 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*
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
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
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.
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.
References


<table>
<thead>
<tr>
<th>Name</th>
<th>Read typea</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIB1</td>
<td>Illumina PE</td>
<td>depth: 50x; read length: 100bp; insert size: 180bp ± 9bp</td>
</tr>
<tr>
<td>LIB2</td>
<td>Illumina MP</td>
<td>depth: 50x; read length: 100bp; insert size: 8000bp ± 400bp</td>
</tr>
<tr>
<td>LIB3</td>
<td>Illumina PE</td>
<td>depth: 50x; read length: 250bp; insert size: 450bp ± 23bp</td>
</tr>
<tr>
<td>LIB4</td>
<td>PacBio CLR</td>
<td>depth: 60x; read accuracy: 0.87 ± 0.03; read length: 11500bp ± 8000bp</td>
</tr>
<tr>
<td>LIB5</td>
<td>PacBio CLR</td>
<td>depth: 10x; read accuracy: 0.87 ± 0.03; read length: 11500bp ± 8000bp</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Assembler</th>
<th>Sequencing strategies used for assembly</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILMN1</td>
<td>ABYSS</td>
<td>LIB1, LIB2 (Illumina-only)</td>
</tr>
<tr>
<td>ILMN2</td>
<td>ALLPATHS-LG</td>
<td></td>
</tr>
<tr>
<td>ILMN3</td>
<td>MaSuRCA</td>
<td></td>
</tr>
<tr>
<td>ILMN4</td>
<td>SGA</td>
<td>LIB1, LIB2 (Illumina-only)</td>
</tr>
<tr>
<td>ILMN5</td>
<td>SOAPdenov2</td>
<td></td>
</tr>
<tr>
<td>ILMN6</td>
<td>SPAdes</td>
<td></td>
</tr>
<tr>
<td>ILMN7</td>
<td>Velvet</td>
<td></td>
</tr>
<tr>
<td>ILMN8</td>
<td>DISCOVAR</td>
<td>LIB3 (Illumina-only)</td>
</tr>
<tr>
<td>PACB</td>
<td>Celera Assembler</td>
<td>LIB4 (PacBio-only)</td>
</tr>
<tr>
<td>HYBR</td>
<td>SPAdes</td>
<td>LIB1, LIB2, LIB5 (Hybrid)</td>
</tr>
</tbody>
</table>

aRead type: PE – Paired-end; MP – Mate pair; CLR: Continuous long read.
<table>
<thead>
<tr>
<th>Nuclear:mito genome ratio</th>
<th>Complete, single contig assembly</th>
<th>Coverage ≥ 99%</th>
<th>20% ≤ Coverage &lt; 99%</th>
<th>Coverage &lt; 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50 (low mitochondrial content)</td>
<td>ILMN1, ILMN6, ILMN8, HYBR</td>
<td>ILMN7</td>
<td>ILMN2, ILMN4, ILMN5, PACB</td>
<td>ILMN3</td>
</tr>
<tr>
<td>1:200 (high mitochondrial content)</td>
<td>HYBR</td>
<td>ILMN6, ILMN7</td>
<td>ILMN1, ILMN8, PACB</td>
<td>ILMN2, ILMN3, ILMN4, ILMN5</td>
</tr>
</tbody>
</table>
### Table 3. Summary of top-ranking assemblies generated in case study III.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Top 2 assemblies</th>
<th>Assembly statistics$^1$</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Scaffold N50 (kb)</td>
<td>Largest scaffold (kb)</td>
<td>Coverage</td>
<td></td>
</tr>
<tr>
<td><strong>D. melanogaster</strong> (137.55 Mb)</td>
<td>PACB</td>
<td>443.8</td>
<td>3584.5</td>
<td>98.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ILMN8</td>
<td>155.0</td>
<td>1007.7</td>
<td>91.8%</td>
<td></td>
</tr>
<tr>
<td><strong>A. thaliana</strong>   (119.15 Mb)</td>
<td>PACB</td>
<td>626.9</td>
<td>2603.0</td>
<td>99.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ILMN8</td>
<td>266.6</td>
<td>2533.4</td>
<td>98.5%</td>
<td></td>
</tr>
<tr>
<td><strong>Pf. falciparum</strong> (23.29 Mb)</td>
<td>ILMN8</td>
<td>222.0</td>
<td>729.9</td>
<td>98.4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ILMN2</td>
<td>28.0</td>
<td>146.2</td>
<td>96.7%</td>
<td></td>
</tr>
</tbody>
</table>

$^1$All statistics shown are after correction using the reference genome.
**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.
Figure 1

**Input**

Reference genome + Experimental design

**Step 1: Simulation**

- ART/pIRS
- Illumina
  - Paired-end (PE) / Mate-pair (MP) short reads
- PacBio
  - Continuous long reads

**Step 2: Quality control**

Quality/adapter trimming

- Trimmomatic (PE)
- NextClip (MP)

Error correction

- Quake (PE)

**Step 3: Assembly**

- **Illumina-only assembly**
  - ABYSS,
  - ALLPATHS-LG,
  - CA,
  - DISCOVAR,
  - MaSuRCA,
  - SGA,
  - SOAPdenovo2,
  - SPAdes,
  - Velvet

- **Hybrid assembly**
  - ALLPATHS-LG,
  - CA,
  - SPAdes

- **PacBio-only assembly**
  - CA

**Step 4: Evaluation**

- QUAST
  - vs. the reference genome
    - Corrected N50 and largest contig, recovered genome fraction, misassemblies, etc.

**Output**

- QUAST evaluation report + Rank of experimental designs

---

Input Reference genome + Experimental design

Step 1: Simulation

- ART/pIRS
- Illumina
  - Paired-end (PE) / Mate-pair (MP) short reads
- PacBio
  - Continuous long reads

Step 2: Quality control

- Quality/adapter trimming
  - Trimmomatic (PE)
  - NextClip (MP)
- Error correction
  - Quake (PE)

Step 3: Assembly

- Illumina-only assembly
  - ABYSS,
  - ALLPATHS-LG,
  - CA,
  - DISCOVAR,
  - MaSuRCA,
  - SGA,
  - SOAPdenovo2,
  - SPAdes,
  - Velvet

- Hybrid assembly
  - ALLPATHS-LG,
  - CA,
  - SPAdes

- PacBio-only assembly
  - CA

Step 4: Evaluation

- QUAST
  - vs. the reference genome
    - Corrected N50 and largest contig, recovered genome fraction, misassemblies, etc.

Output

- QUAST evaluation report + Rank of experimental designs
Figure 2

- **Zymoseptoria tritici**
  - Coverage (%): 100
  - Corrected scaffold N50 (kb): 100
  - Corrected largest scaffold (kb): 1000

- **Pseudocercospora fijiensis**
  - Coverage (%): 90
  - Corrected scaffold N50 (kb): 90
  - Corrected largest scaffold (kb): 900

- **Cenococcum geophilum**
  - Coverage (%): 80
  - Corrected scaffold N50 (kb): 80
  - Corrected largest scaffold (kb): 800

**Protocols**
- ILMN2
- ILMN8
- HYBR
- PACB

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