de novo assembly and population genomic survey of natural yeast isolates with the Oxford Nanopore MinION sequencer

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Abstract

Background: Oxford Nanopore Technologies Ltd (Oxford, UK) have recently commercialized MinION, a small single-molecule nanopore sequencer, that offers the possibility of sequencing long DNA fragments from small genomes in a matter of seconds. The Oxford Nanopore technology is truly disruptive, it has the potential to revolutionize genomic applications due to its portability, low-cost, and ease of use compared with existing long reads sequencing technologies. The MinION sequencer enables the rapid sequencing of small eukaryotic genomes, such as the yeast genome. Combined with existing assembler algorithms, near complete genome assemblies can be generated and comprehensive population genomic analyses can be performed.

Results: Here, we resequenced the genome of the *Saccharomyces cerevisiae* S288C strain to evaluate the performance of nanopore-only assemblers. Then we *de novo* sequenced and assembled the genomes of 21 isolates representative of the *S. cerevisiae* genetic diversity using the MinION platform. The contiguity of our assemblies was 14 times higher than the Illumina-only assemblies and we obtained one or two long contigs for 65% of the chromosomes. This high contiguity allowed us to accurately detect large structural variations across the 21 studied genomes.

Conclusion: Because of the high completeness of the nanopore assemblies, we were able to produce a complete cartography of transposable elements insertions and inspect structural variants that are generally missed using a short-read sequencing strategy. Our analyses show that the Oxford Nanopore technology is already usable for de novo sequencing and assembly; however non-random errors in homopolymers require polishing the consensus using an alternate sequencing technology.

Keywords: *de novo* assembly; Nanopore sequencing; Oxford Nanopore; MinION device; genome finishing; structural variations; transposable elements

Background

Today, long-read sequencing technology offers interesting alternatives to solve genome assembly difficulties and improve the completeness of genome assemblies, mostly in repetitive regions [1] where short-read sequencing has failed. Microbial or small eukaryotic genomes could now be assembled using Oxford Nanopore [2] or Pacific Biosciences reads alone [3, 4] or in combination with short but high quality reads [5-7]. Application of the single-molecule real-time (SMRT) sequencing platform to large complex eukaryotic genomes demonstrated the possibility of considerably improving genome assembly quality [8, 9]. Similar improvements were also accomplished using the 10x Genomics platform, and its application to the human genome produced encouraging results [10-12] and showed the importance of obtaining long and high-quality reads.

The most used sequencing technologies are based on the synthesis of new DNA strands, including the Illumina and Pacific Biosciences technologies [13]. These sequencing technologies based on optical detection of nucleotide incorporations are often commercialized through large-sized and expensive instruments. For example, the cost of the commercially available Pacific Biosystems RS II instrument is high and the infrastructure and implementation needs make it inaccessible to large sections of the research community. This year Oxford Nanopore Technologies Ltd (ONT, Oxford, UK) commercialized MinION, a single-molecule nanopore sequencer that can be connected to a laptop through a USB interface [14, 15]. This system is portable (close to the size of a harmonica) and low-cost (currently USD 1,000 for the instrument). The MinION technology is based on an array of nanopores embedded on a chip that detects consecutive 6-mers of a single-strand DNA molecule by electrical sensing [16-19]. In addition to its small size and low price, this new technology has several advantages over the older technologies. Library construction involves a simplified method, no amplification step is needed, and data acquisition and analyses occur

in real time [20]. Library preparation can be performed in two ways: (i) a 10-minute library preparation based on an enzymatic method for '1D' sequencing (sequencing one strand of the DNA) or (ii) a library preparation based on ligation for '2D' sequencing (sequencing both the template and complement strands of the DNA). In the 2D sequencing mode, the two strands of a DNA molecule are linked by a hairpin and sequenced consecutively. When the two strands of the molecule are read successfully, a consensus sequence is built to obtain a more accurate read (called 2D read). Otherwise only the template or complement strand sequence is provided (called 1D read).

Here, we sequenced the genomes of 22 *Saccharomyces cerevisiae* isolates to determine if the MinION system could be used in population genomic projects that require a deeper view of the genetic variation landscape. Even if the throughput of MinION was still heterogeneous, we were able to perform the sequencing in a reasonable time using six MinION devices (less than 2 days per strain). First, we resequenced the *Saccharomyces cerevisiae* S288C reference genome using a nanopore long-read sequencing strategy to evaluate recent assembly methods. We generated a complete benchmark of the assembly structures, as well as the completeness of complex regions. Next, we selected 21 strains of *S. cerevisiae* that were genetically diverse, based on preliminary results of the 1002 Yeast Genomes Project a large-scale short-read resequencing project (http://1002genomes.u-strasbg.fr/). The genomes of these 21 strains were *de novo* sequenced and assembled with Nanopore long-reads to have a better insight into the variation of their genomic architecture. We obtained near complete assembly, in terms of genes, as well as transposable elements and telomeric regions. The most contiguous assembly produced a single contig per chromosome, except for chromosomes 3 and 12, the latter containing the large repeated rDNA cluster.

Results

MinION data evaluation

We first sequenced the S288C genome by doing 11 MinION Mk1 runs with the R7.3 chemistry. On average, a 48-hours run produced more than 200 Mb of sequence, and the best run throughput was 400 Mb. Two 2D library types with 8 kb and 20 kb mean fragmentation sizes were used. They led to nearly 360,000 reads with a cumulative length of approximately 2.3 Gb and 63% of the nucleotides were in 2D reads, which represented a 187x and 118x genome coverage for 1D and 2D reads, respectively. Template reads had a median length of 8.9 kb while 2D reads had a median length of 7.7 kb. All sequencing reads were aligned to the S288C reference genome using BWA [21] to assess their quality. We successfully aligned 95.6% of the 2D reads with an average error rate of 17.2% (**Figure 1a**). ONT tagged high-quality 2D reads as "2D pass" reads (reads with an average per-base quality higher than 9), and 99.7% of the 2D pass reads were aligned to the reference genome with an average error rate of 12.2%. We then parsed the alignment files to search for errors in stretches of the same nucleotide (homopolymers). About 85% of A, T, C, and G homopolymers of size 2 were present correctly in the reads. This percentage decreased rapidly to 65% for homopolymers of size 4 for A and T homopolymers and to 70% for C and G homopolymers. For size 7 homopolymers, it was 30% for A and T homopolymers and 35% for C and G homopolymers (**Figure S1a**).

We also sequenced the S288C genome using the R9 chemistry, the recently released version of the pore. We obtained approximately 1 Gb of reads; 568 Mb were 2D reads, which represents a 85x coverage with 1D reads and a 47x coverage with 2D reads. The mean 2D length was 6.1 kb. We aligned 82.1% of the 1D reads with a mean identity percentage of 82.8% and 94.3% of the 2D reads with a mean identity percentage of 85.2% (**Figure 1b**). As we did with the R7.3 reads, we also searched for errors in homopolymers (**Figure S1b**). The

numbers of correct A, T, C, and G homopolymers started at about 90% for size equal to 2, then decreased to 75% for A and T homopolymers of size 4 and to 60% for the C and G homopolymers. For size 7 homopolymers, it was 32% for A, T, and C homopolymers and 35% for G homopolymers.

Comparison of Nanopore-only assemblers

We tested Canu [22], Miniasm [23], SMARTdenovo [24] and ABruijn [25] with different subset of 1D, 2D, and 2D pass reads (**Supplementary File 2 and Table S1**) and kept the most contiguous assembly for each software.

With Canu, the assembly with the higher N50 was obtained with the whole set of 2D pass reads (67x coverage). The assembly was composed of 37 contigs with a cumulative length of 12 Mb and seven chromosomes were assembled in one or two contigs. After aligning the contigs to the S288C reference genome using Quast [26], we detected a high number of deletions (120,365), which were often localized in homopolymers (58%). As a consequence, only 454 of the 6,243 genes found in the assembly were insertion/deletion (indel)-free (**Table S2**). With Miniasm, the most contiguous assembly was obtained using the 2D reads corrected by Canu, which represented coverage of approximately 108x. The Miniasm assembly was composed of 28 contigs with a cumulative length of 11.8 Mb, and 13 chromosomes were assembled in one or two contigs. The Miniasm consensus sequence contained the higher number of mismatches and indels (**Table S2**). With SMARTdenovo, 30x of the longest 2D reads produced the assembly with the highest contiguity. It was composed of 26 contigs, with a total length of 12 Mb, and 14 chromosomes were assembled in one or two contigs. The SMARTdenovo assembly better covered the reference genome (>99%) and contained the highest number of genes (98.8% of the 6,350 S288C genes), but the Quast output again revealed a high number of deletions (128,050). With ABruijn, we obtained the assembly with the highest N50 when using all the 2D reads as input, which represented coverage of

approximately 120x. The assembly contained 23 contigs with a cumulative length of 11.9 Mb, and 14 chromosomes were assembled in one or two contigs (**Table S2**).

Next, we aligned the assemblies (Canu, Miniasm, SMARTdenovo, and ABruijn) to the S288C reference genome using NUCmer [27], and visualized the alignments with mummerplot (**Figures S2, S3, S4 and S5**). We also examined the coordinates of the alignments to search for chimera. We did not detect any chimeric contigs in the Canu, Miniasm, or SMARTdenovo assemblies; however, we did find some in the ABruijn assembly. Three chimeric contigs in the ABruijn assembly showed links between chromosomes 3 and 13 (first contig), chromosomes 3 and 2 (second contig), and chromosomes 10 and 2 (third contig). To verify that the portions of these contigs were effectively chimeric, we back aligned the Nanopore reads to the assembly and could not find any sequence that validated these links. Unsurprisingly, these three chimeric contigs were fused at Ty1 transposable element locations.

The alignment of each assembly to the reference genome showed that neither Canu, Miniasm, nor SMARTdenovo could assemble the mitochondrial (Mt) genome completely. Because ABruijn was the only assembler to assemble the complete Mt genome sequence, we decided to use it to assemble the Mt DNA of the remaining 21 yeast strains (see below).

Generally, long reads allow tandem duplicated genes to be resolve, as for instance the *CUP1* and *ENA1-2* gene families. We compared the maximum number of copies found in the Nanopore reads and the estimated number of copies based on Illumina reads coverage of these two tandem-repeated genes with the number of copies of these two genes in the four assemblies (**Table S3**). After aligning the paired-end reads to the reference sequence and computing of the coverage, we estimated that *CUP1* and *ENA1-2* were present in seven and four copies, respectively. The maximum numbers of copies of these genes in a single Nanopore read were eight for *CUP1* and five for *ENA1-2*. The numbers of copies of *CUP1*

and *ENA1-2* were, respectively, nine and three in the Canu assembly, seven and two in the Miniasm assembly and seven and four in the SMARTdenovo and ABruijn assemblies.

The number of indels in each assembly was considerably high for each assembler. Thus, we tested Nanopolish [2], the most commonly used Nanopore-only error corrector. We used the SMARTdenovo assembly, which was the most continuous and gene-rich assembly and all 2D reads for this test. After the error correction step, the cumulative length of the contigs increased to 12.2 Mb and the N50 increased to 783 kb (at best it was 924 kb for the reference genome). The number of mismatches, insertions and deletions decreased to 1,930, 7,707, and 17,445 respectively. The number of genes increased to 6,273 complete and 2,590 without an indel (**Table 1**).

Although all metrics were improved, the number of indels was still too high, especially in the coding regions of the genes. We decided to polish all assemblies with 2x250bp Illumina paired-end reads at 300X genome coverage, using Pilon [28], to verify if the general quality of the assembly improved. The polishing step increased the N50 of each assembly, and the maximum of 816 kb was obtained with the ABruijn assembly. Pilon reduced the number of errors of each assembly, and the Canu and ABruijn assemblies had the best base quality with about 16 mismatches (15.85 and 17.88 for Canu and ABruijn respectively) and 22 indels (22.49 and 21.76 for Canu and ABruijn respectively) per 100 kb. The SMARTdenovo assembly contained the highest number of complete genes (6,266) and the Canu assembly contained the highest number of genes without any indels (5,921) (**Table 2**). Furthermore, we estimated the impact of the input coverage used to polish the consensus. We performed successive polishing by using subsets of Illumina reads (ranging from 25X to 300X genome coverage). We observed similar results in terms of number of mismatches and indels, regardless of the input coverage. (**Figure S6**).

Finally, we evaluated the composition of each assembly for various elements (genes, repeated elements, centromeres and telomeric regions). We also generated an Illumina-only assembly using Spades assembler [29] to compare the number of features found in each assembly. All the assemblies contained nearly the same number of centromeres (120 bp regions in the reference genome assembly) and genes (**Figure 2**). The Nanopore assemblies contained more complete genes than the Illumina one, however genes without indels are more frequent in the Illumina-only assembly although nanopore assemblies were polished using Illumina reads. even between 45 and 50 Long Terminal Repeat (LTR) retrotransposons (average size of 5.8 kb), while the Illumina-only assembly contained only one. The smallest number of telomeres (three) was found in the ABruijn assembly, while nine, 18, 13, and 14 telomeres were found in the Illumina, Canu, Miniasm, and SMARTdenovo assemblies, respectively. The Illumina-only assembly contained five telomeric repeats (average size 100 bp), while the Nanopore-only assemblies contained between six and nine telomeric repeats. The ABruijn assembly contained the same number of genes encoded by the mitochondrial genome as the reference sequence because it was the only assembler to fully assemble the Mt genome.

S288C assemblies with R9 data

The R9 version of the pore was released too late for us to use it to sequence all the natural *S. cerevisiae* isolates. However, we did produce some data to compare the R7.3 and R9 assemblies. Because SMARTdenovo produced the best results (higher continuity and higher gene content), we used it to assemble the R9 data generated from the S288C strain. We input four different read datasets: all 1D and 2D reads, only 2D reads, 30x of the longest 2D reads or 30x of the longest 1D and 2D reads **(Table S4)**.

This time, the 30x of the longest 1D and 2D reads dataset gave the best results. Indeed, the contiguity of the assembly increased, and the number of contigs decreased from 26 with the R7.3 assembly to 23 with the R9 assembly. The number of indels also decreased from

133,676 with the R7.3 version to 95,012 with the R9 version. A direct consequence of using the R9 version was that almost all the genes were found, and 6,302 of the 6,350 known genes were complete and 1,226 did not contain any indels.

Sequencing and assembly of the genomes of the 22 yeast strains

To explore the variability of the genomic architecture within *S. cerevisiae*, 21 natural isolates were sequenced in addition to the S288C reference genome using the same strategy, namely, a combination of long Nanopore and short Illumina reads. Sequenced isolates were selected to include as much diversity as possible in terms of global locations (including Europe, China, Brazil, and Japan), ecological sources (such as fermented beverages, dairy products, trees and fruit soil), as well as genetic variation highlighted in the frame of the extensive resequencing 1002 Yeast Genomes project (http://1002genomes.u-strasbg.fr/) (**Table S5**). Among these isolates, the nucleotide variability was distributed across 491,076 segregating sites and the genetic diversity, estimated by the average pairwise divergence (π), was 0.0062, which is close to what is observed for the whole species [30].

A total of 78 MinION Mk1 runs were performed and the highest throughput we obtained was 650 Mb (1D and 2D reads). This led to 1.4 million of 2D reads with a cumulative length of 12 Gb. We obtained 2D coverage that ranged from 22x to 115x (**Figure S7**) among the strains with a median read length of approximately 5.4 kb and a maximum size of 75 kb (**Figure S8**). In general, three runs or less were sufficient to obtain the expected coverage. Next, for each strain, we gave varying coverages of the longest 2D reads (**Table S6**) as input to SMARTdenovo and retained the most contiguous assembly. These assemblies were then given as input to Pilon for a polishing step with around 300x of Illumina paired-end reads (each strain was individually sequenced using the Illumina technology). After polishing, we obtained a median number of contigs of 27.5 (**Table 3**), the minimum number was for the CEI strain (18 contigs) and the maximum was for the BAM strain (105 contigs). The median

cumulative length was 11.93 Mb and ranged from 11.83 Mb for the ADQ strain to 12.2 Mb for the CNT strain. The median N50 contig size was 593 kb and varied from 201 kb for the CIC strain to 896 kb for the ADQ strain. The L90 varied from 14 for the BCN, CEI, and CNT strains, to 72 for the BAM strain with a median equal to 19.5.

To assemble the mitochondrial (Mt) genome, we used all the 2D reads as input to ABruijn. As a result, we obtained an assembly for each strain and extracted the Mt genome after mapping the contigs against the reference Mt genome. As was the case for the chromosomes, we used Pilon with Illumina paired-end reads to obtain a corrected consensus sequence.

Transposable elements

The availability of high quality assemblies allowed us to establish an extensive map of the transposable elements (TEs) to obtain a global view of their content and positions within the 21 natural yeast isolates (**Figure 3**). Using a reference sequence for each of the five known TE families in yeast (namely Ty1 to Ty5), we mapped the TEs in each assembled genome. Among the 50 annotated TEs in the S288C reference genome, 47 were detected at the correct chromosomal locations in our assembly but three Ty1 locations were not recovered. Seven additional Ty1 elements were found at unannotated sites, three of them have already been detected in the reference genome [31]. These results attest to the high accuracy of our assembly strategy for TE detection and localization. Among the 22 isolates, the TE content was highly variable (**Table 4**), ranging from five to 55 elements, with a median value of 15. While the frequency of the Ty4 and Ty5 elements was clearly low in all the isolates (up to four and two elements, respectively), the Ty1, Ty2, and Ty3 elements were found in most of the isolates. The most abundant TEs were Ty1 and Ty2, except in the Chinese BAM isolate, in which 12 Ty3 elements were detected. As already described [32], the pattern of insertion of these mobile elements is either specific to a given isolate, or shared by only a small number of isolates (mostly two or three). However, four insertion hotspots have been highlighted (shared

by seven or more isolates) on chromosomes 2, 3, and 9. The shared insertion hotspots were generally not specific to a specific Ty family, except for the hotspot located on a subtelomeric region of the chromosome 3, which was specific to Ty5.

Structural variations

Structural variations such as copy number variants, large insertions and deletions, duplications, inversions and translocations are of great importance at the phenotypic variation level [33]. Compared with single nucleotide polymorphism (SNPs) and small indels, these variants are usually more difficult to identify, in particular because resequencing strategies have until recently focused mainly on the generation of short reads and reference-based genome analysis. Nanopore long reads sequencing data allow the copy numbers of tandem genes to be determined. As a testbed, we focused on two loci that are known to contain multi-copy genes, namely *ENA* and *CUP1*. *ENA* genes encode plasma membrane Na^+-ATPase exporters, which play a role in the detoxification of Na+ ions in *S. cerevisiae*. *CUP1* genes encode metallothioneins, which bind copper and are involved in resistance to copper exposure by amplification of this locus. To determine the degree of divergence among the 21 strains, we searched for the numbers of copies of the *CUP1* and *ENA*, two tandem-repeated genes in the assemblies (**Table 5**). For this purpose, we extracted the corresponding sequence from the S288C reference genome and aligned it to the assemblies of each strain. As expected and already reported [34], the copy numbers of *ENA1* and *CUP1* varied greatly across the strains. We found that the copy numbers of *ENA* genes in the 21 isolates ranged from 1 in 12 of the genomes to five in the BHH strain (**Table 5**). The copy numbers of *CUP1* genes fluctuated even more, ranging from one to 10 copies in the ABH and AEG strains. We also determined the fitness of the 21 isolates in the presence of $CuSO_4$ and observed a correlation between the number of *CUP* genes and the resistance of the strain to high concentration of $CuSO_4$ (**Figure S9**).

Besides copy number variants, we also focused on larger structural variants, such as translocations and inversions, because our highly contiguous assemblies allowed us to investigate these events. We aligned the polished assemblies of the 21 strains to the reference genome using NUCmer and inspected the alignments with the mummer software suite to search for structural variations. We detected 29 translocations and four inversions within the assemblies of 17 strains (**Table 6**). The median length of an inversion was 94 kb and their breakpoints were located mostly in intergenic regions. It is well recognized that SVs might play a major role in the genetic and phenotypic diversity in yeast [35, 36]. However, up to now, it was impossible to assemble and have an exhaustive view of the SVs content in any *S. cerevisiae* natural isolates. Indeed, short-read sequencing approaches are not suitable for SVs studies because they results in a high number of false positive as well as false negative detected events.

Among the detected events, one translocation detected between chromosomes 5 and 14 in the ABH isolate and another translocation between chromosomes 7 and 12 in the AVB isolate have already been described and confirmed in a reproductive isolation study in *S. cerevisiae* [35]. A deeper investigation of our assemblies highlighted the presence of full-length Ty transposons at some junctions of the translocation events. For example, the complex Ty-rich junctions of the translocation between the chromosomes 7 and 12 in the ABH isolate was in complete accordance with previously reported results [35]. Our results underline the high resolution of the constructed assemblies, and show that complex events, such as translocations, can be detected accurately with our strategy. Among the 22 isolates, six were devoid of translocation events whereas the other 16 carries one to four such structural rearrangements compared to the reference.

However, several limitations can be highlighted for these detections. Contrary to expectations, no translocation that specifically affected subtelomeric regions was identified, underlining the

difficulty of discriminating regions that are variable and contain a large number of repeated segments. Moreover, the detection accuracy is highly dependent on the completeness of the assembly because, if translocation breakpoints are located on contigs boundaries, they will not be detectable.

Mitochondrial genome variation

The ABruijn assembler allowed the construction of a single contig corresponding to the Mt genome for each isolate. To assess the quality of the assemblies, we aligned the polished S288C Mt contig to the reference sequence (GenBank: KP263414). Only four SNPs and few indels, representing 15 bp of cumulative length, were detected. For all but two natural isolates, all the Mt genes (eight protein coding genes, two rRNA subunits and 24 tRNAs) were conserved and syntenous. The Mt genomes of the two remaining isolates (CNT and CFF) contained one and two repeated regions covering a total of 6.5 and 8 kb, respectively. In the CNT, the repeated region was in the *COX1* gene and affected its coding sequence. In the CFF isolate, the *COX1*, *ATP6,* and *ATP8* genes would have been tandemly duplicated. However, because we could not identify reads that clearly covered the repeated regions and then confirmed the structural variations, we excluded these two Mt genome assemblies from our dataset.

The sizes of the 20 considered assemblies ranged from 73.5 to 86.9 kb, which is close to the size reported previously [37]. The differences in size between the assemblies can mainly be attributed to the intron content of the *COX1* and *COB* genes (from two to eight introns in *COX1* and from two to six introns in *COB*). These variations lead to extensive gene length variability ranging from 5.7 kb to 14.9 kb for *COX1* and from 3.2kb to 8.6 kb for *COB*, while the coding sequences of these 2 genes were exactly the same length among the 20 isolates. Intergenic regions also accumulate many small indels, including those that affect the interspersed GC-clusters, and a few large indels that sometimes correspond to variable

hypothetical open reading frames (ORFs), leading to sizes that range from 51.6 to 58 kb. To a lesser extent, the 21S rRNA gene is also subjected to size variation that ranges from 3.2 to 4.4 kb.

Discussion

One of the major advantages of the Oxford Nanopore technology is the possibility of sequencing very long DNA fragments. In our analyses, we obtained 2D reads up to 75 kb in length, indicating that the system was able to read without interruption a flow of at least 150,000 nucleotides. Furthermore, the results of this analysis indicate that the error rate of the ONT R7.3 reads was in the range that is obtained using existing long-read technologies (i.e, about 15% for 2D reads). However, the errors are not random and they significantly impact stretches of the same nucleotides (homopolymers), which seems to be a feature inherent to the ONT sequencing technology. Because the pore detects six nucleotides at a time, segmentation of events is problematic in genomic regions with homopolymers longer than six bases [38]. With the current R7.3 release, homopolymers are prone to base deletion (representing 66% of the errors observed in homopolymers). It may be improved with a steadier passing speed through the pore or by increasing the speed of the molecule through the pore. In the same way, the basecaller algorithm could be optimized to increase the accuracy per base. ONT have recently reported several changes, including a fast mode (250 bp/second instead of 70 bp/second with R7.3 chemistry) and new basecaller software based on neural networks. These new features are incorporated in the R9 version of MinION. We performed R9 experiments, and observed a significant decrease in the error rate (with 1D and 2D reads, **Figure 1**). Using this new release, homopolymers were more prone to base insertions (representing 63% of the errors observed in homopolymers). Systematic errors are problematic for genome assembly

because they lead to the construction of less accurate consensus sequences. Furthermore, indels negatively impact gene prediction because they can create frameshifts in the coding regions of genes. We concluded that nanopore-only assemblies are difficult to use for analysis at the gene level unless they are polished. However, polishing based only on nanopore reads was not sufficient because although it reduced the number of indels by more than seven times, we still had about 3,700 genes that were affected by potential frameshifts. The recently developed R9 chemistry greatly improved the overall quality of the consensus sequences, because starting with only 45x of 2D reads we obtained an assembly with the same contiguity but with a decrease of nearly 30% in the number of indels (95,012 compared with 133,676). We consider that the ONT sequencing platform will evolve in the coming years to produce high quality long reads. Until then, a mixed strategy using high quality short reads remains the only way to obtain high quality consensus sequences as well as a high level of contiguity. Indeed, for the assembly of repetitive regions, the nanopore-only assemblies outperformed the short-reads assemblies.

Our benchmark of nanopore-only assemblers shows that unfortunately a single "best assembler" does not exist. Canu reconstructed the telomeric regions better and provided a consensus of higher quality than Miniasm and SMARTdenovo. ABruijn seemed to produce the most continuous assembly but some of the contigs were chimeric. However, ABruijn was the only assembler to fully assemble the mitochondrial genome, and that is why we chose it to assemble the Mt genomes of the 22 yeast strains. SMARTdenovo provided good overall results for repetitive regions, completeness, contiguity, and speed. It was the most appropriate choice to assemble the genome of all the yeast strains even if its major drawback was the absence of the Mt genome sequence among the contig output.

The high contiguity of the 22 nanopore-only assemblies allowed us to detect transposable element insertions and to provide a complete cartography of these elements. Ty1 was the most

abundant element and it was spread across the entire genome. Chromosome 12 was always the most fragmented in our assemblies due to the presence of the rDNA cluster (around 100 copies in tandem). Furthermore, we easily identified known translocations (between chromosomes 5 and 14 in the ABH isolate and between chromosomes 7 and 12 in the AVB isolate). The high contiguity of the assemblies seemed to be limited by the read size rather than the error rate. Work is still needed to prepare high-weight molecular DNA, enriched in long fragments. The yeast genomes were successfully assembled with 8 kb and 20kb fragment-sized libraries, but more complex genomes will require longer reads.

Methods

DNA extraction

Yeast cells were grown on YPD media (1% yeast extract, 2% peptone and 2% glucose) using liquid culture or solid plates. Total genomic DNA was purified from 30 ml YPD culture using Qiagen Genomic-Tips 100/G and Genomic DNA Buffers as per the manufacturer's instructions. The quantity and quality of the extracted DNA were controlled by migration on agarose gel, spectrophotometry (NanoDrop ND-1000), and fluorometric quantification (Qubit, ThermoFisher).

Illumina PCR-free library preparation and sequencing

DNA (6 µg) was sonicated to a 100 to 1500 bp size range using a Covaris E210 sonicator (Covaris, Woburn, MA, USA). Fragments were end-repaired using the NEBNext® End Repair Module (New England Biolabs, Ipswich, MA, USA) and 3′-adenylated with the NEBNext dA-Tailing Module. Illumina adapters were added using the NEBNext Quick Ligation Module. Ligation products were purified with AMPure XP beads (Beckmann Coulter Genomics, Danvers, MA, USA). Libraries were quantified by qPCR using the KAPA Library Quantification Kit for Illumina Libraries (KapaBiosystems, Wilmington, MA, USA) and library profiles were assessed using a DNA High Sensitivity LabChip kit on an Agilent

Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Libraries were sequenced on an Illumina MiSeq or a HiSeq 2500 instrument (San Diego, CA, USA) using 300 or 250 base-length read chemistry in a paired-end mode.

Nanopore 20 kb libraries preparation

MinION sequencing libraries were prepared according to the SQK-MAP005 or SQK-MAP006-MinION gDNA Sequencing Kit protocols. Six to 10 µg of genomic DNA was sheared to approximately 20,000 bp with g-TUBE (Covaris, Woburn, MA, USA). After clean-up using 0.4x AMPure XP beads, sequencing libraries were prepared according to the SQK-MAP005 or SQK-MAP006 Sequencing Kit protocols, including the PreCR treatment (NEB, Ipswich, USA) for the SQK-MAP005 protocol or the NEBNext FFPE DNA repair step (NEB, Ipswich, USA) for the SQK-MAP006 protocol.

Nanopore 8 kb libraries preparation

MinION sequencing libraries were prepared according to the SQK-MAP005 or SQK-MAP006-MinION gDNA Sequencing Kit protocols. Two µg of genomic DNA was sheared to approximately 8,000 bp with g-TUBE. After clean-up using 1x AMPure XP beads, sequencing libraries were prepared according to the SQK-MAP005 or SQK-MAP006 Sequencing Kit protocol, including the PreCR treatment for the SQK-MAP005 protocol or the NEBNext FFPE DNA repair step for the SQK-MAP006 protocol.

Nanopore Low input 8 kb libraries preparation

The following protocol was applied to some samples (**Supplementary File 3**). Five hundred ng of genomic DNA was sheared to approximately 8,000 bp with g-TUBE. After clean-up using 1x AMPure XP beads and the NEBNext FFPE DNA repair step, 100 ng of DNA was prepared according to the Low Input Expansion Pack Protocol for genomic DNA.

MinION™ flow cell preparation and sample loading

The sequencing mix was prepared with 8 µL of the DNA library, water, the Fuel Mix and the Running buffer according to the SQK-MAP005 or the SQK-MAP006 protocols. The sequencing mix was added to the R7.3 flowcell for a 48 hours run. The flowcell was then reloaded three times according to the following schedule: 5 hours (4 µL of DNA library), 24 hours (8 µL of DNA library) and 29 hours (4 µL of DNA library). Regarding the Low Input libraries, the flowcell was loaded and then reloaded after 24 hours of run time with a sequencing mix containing 10 µL of the DNA library (**Supplementary File 3**).

MinION® sequencing and reads filtering

Read event data generated by MinKNOW™ control software (version 0.50.1.15 to 0.51.1.62) were base-called using the Metrichor™ software (version 2.26.1 to 2.38.3). The data generated (pores metrics, sequencing, and base-calling data) by MinION software were stored and organized using a Hierarchical Data Format (HDF5). Three types of reads were obtained: template, complement, and two-directions (2D). The template and complement reads correspond to sequencing of the two DNA strands. Metrichor combines template and complement reads to produce a consensus (2D) sequence [39]. FASTA reads were extracted from MinION HDF5 files using poretools [40]. To assess the quality of the MinION reads, we aligned reads against the *S. cerevisiae* S288C reference genome using the LAST aligner (version 588) [41]. Because the MinION reads are long and have a high error rate we used a gap open penalty of 1 and a gap extension penalty of 1.

Illumina reads processing and quality filtering

After the Illumina sequencing, an in-house quality control process was applied to the reads that passed the Illumina quality filters. The first step discards low-quality nucleotides (Q<20) from both ends of the reads. Next, Illumina sequencing adapters and primers sequences were removed from the reads. Then, reads shorter than 30 nucleotides after trimming were discarded. These trimming and removal steps were achieved using in-house-designed

software based on the FastX package [42]. The last step identifies and discards read pairs that mapped to the phage phiX genome, using SOAP [43] and the phiX reference sequence (GenBank: NC_001422.1). This processing resulted in high-quality data and improvement of the subsequent analyses.

Assembler evaluation

To determine the assembler to use on the *de novo* sequenced 22 yeast strains, tests were conducted on S288C, the only *S. cerevisiae* strain for which there is an established reference genome. We used different subsets of the reads as input to Canu (github commit ae9eecc), Miniasm (github commit 17d5bd1), SMARTdenovo (github commit 61cf13d), and ABruijn (github commit dc209ee), four assemblers that can take advantage of long reads. These subsets consisted of varying coverages of 1D, 2D, 2D pass reads, which are 2D reads that have an average quality greater than nine, and reads corrected by Canu. Canu was executed with the following parameters: genomeSize=12m, minReadLength=5000, mhapSensitivity=high, corMhapSensitivity=high, errorRate=0.01 and corOutCoverage=500. Miniasm was run with the default parameters indicated on the github website. SMARTdenovo was executed with the default parameters and –c 1 to run the consensus step. ABruijn was run with default parameters. After the assembly step, we polished each set of contigs with Pilon (version 1.1.12) using 300X of Illumina 2x250 bp paired-end reads. Assemblies were aligned to the S288C reference genome using Quast in conjunction with the GFF file of S288C to detect assembly errors, and complete and partial genes. We also visualized the alignments using mummerplot to detect chimeric contigs.

Assembly of the genome of the 22 yeast strains

The 22 genomes were assembled by giving varying coverages, going from 10X to 50X, of the longest 2D reads as input to SMARTdenovo with the default parameters and –c 1 to run the consensus step. Then, for each strain, the most contiguous assembly (based on the N50 and

the number of contigs) was polished using ~300X of 2x250bp Illumina paired-end reads (each yeast strain was sequenced separately beforehand).

Genes and transposons detection

To detect genes and transposons in the assemblies, we extracted the corresponding sequences from the reference genome. We then mapped these elements to the assemblies using the Last aligner. Only alignments that showed more than 80% identity over at least 90% of the sequence length were retained and considered as a match. We used a similar procedure to count the maximum number of gene in the Nanopore reads dataset, the only modification was that the percentage identity had to be at least 70% to account for the high error rate of the reads. To estimate the number of copies in the Illumina reads, we aligned paired-end reads to the reference genome with BWA aln and then computed the coverage using samtools mpileup algorithm [44] and divided the number we obtained for each region of interest by the median coverage of the corresponding chromosome.

Feature number estimation

We generated an Illumina-only assembly using Spades version v3.7.0 with default parameters and compare the completeness of this assembly to the nanopore-only assemblies. To estimate the number of features across all S288C assemblies, we aligned each post-polishing consensus sequence to the S288C reference genome using NUCmer. Only the best alignments were conserved by using the *delta-filter -1* command. Next, we used the bedtools suite [45] with the command *bedtools intersect -u -wa -f 0.99* to compare the alignments to the reference GFF file. Finally, we counted the number of features of our interest.

Circularization of mitochondrial genomes

To circularize the Mt genomes, we split the contig corresponding to the Mt sequence in each strain into two distinct contigs. Then, we gave the two contigs as input to the minimus2 [46] tool from the AMOS package. As a result, we obtained a single contig that did not contain the

overlap corresponding to the circularization zone. Finally, to start the Mt sequence of all isolates at the same position as the reference, we mapped each Mt sequence to the reference using NUCmer. The *show-coords* command allowed us to identify the position in the Mt sequences of all the strains that corresponded to the first position of the reference Mt genome.

Declarations

Availability of Data and Materials

The 22 genome assemblies are freely available at http://www.genoscope.cns.fr/yeast. The Illumina and MinION data are available in the European Nucleotide Archive under accession number ERP016443.

Abbreviations

ONT: Oxford Nanopore Technology; SMRT: Single-Molecule Real-Time Sequencing; USB: Universal Serial Bus; Mt: Mitochondrial; LTR: Long Terminal Repeat; SNP: Single Nucleotide Polymorphism; ORF: Open Reading Frame; MAP: MinION Access Programme.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests. Oxford Nanopore Technologies Ltd contributed to this study by providing some of the R9 reagents free of charges. BI, SD, CCR, AL, SE, PW and JMA are part of the MinION Access Programme (MAP).

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Author's contributions

CCA extracted the DNA. EP, OB, CCR and AL optimized and performed the sequencing. BI, AF, LDA, SF, SD, SE and JMA performed the bioinformatic analyses. BI, AF, JS and JMA wrote the article. GL, PW, JS and JMA supervised the study.

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Additional files

All the supporting data are included as a three additional files: a first one which contains Figures S1-S9 and Tables S1-S6 and two excel files which contain the metrics of all assemblies generated in this study and the description of each MinION run.

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Figures

Figure 1: Identity distribution of Nanopore reads. Percent identity of the aligned MinION 1D (red bars) and 2D (green bars) reads. The MinION reads were aligned using LAST software. **a.** R7.3 chemistry **b.** R9 chemistry

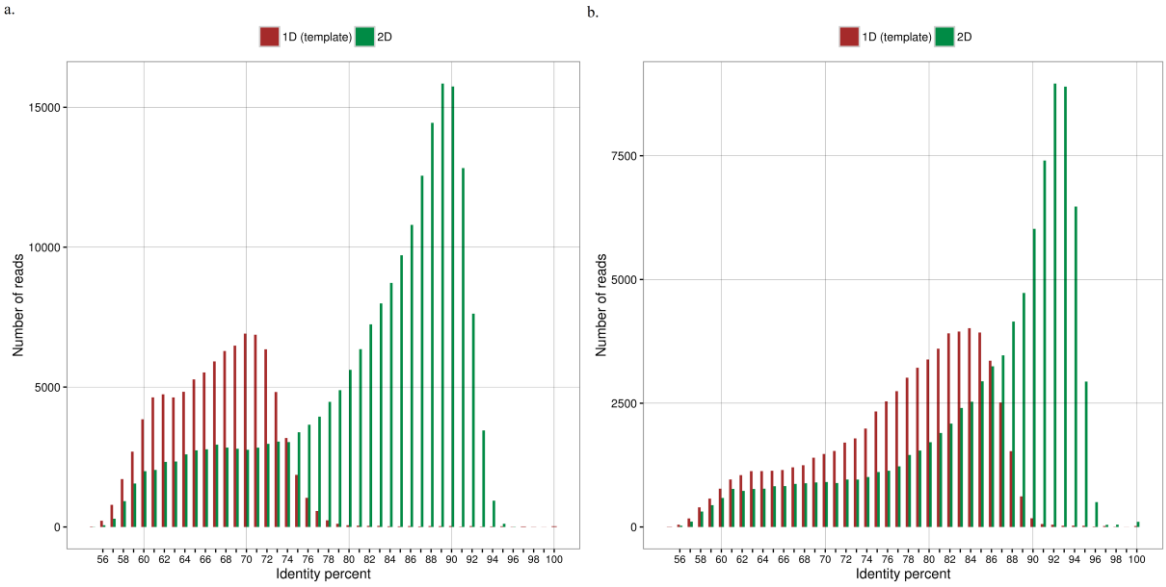

Figure 2: Feature composition of the S288C assemblies, assembly and quality metrics and assembler running statistics. The feature content of the best S288C assemblies for each assembler is shown in the left part of the figure. The feature composition was obtained by aligning each assembly to the S288C reference genome. Assembly and quality metrics for each assembly, obtained by using Quast, are shown in the middle part of the figure. The running time and the memory usage of each assembler are shown in the right part of the figure.

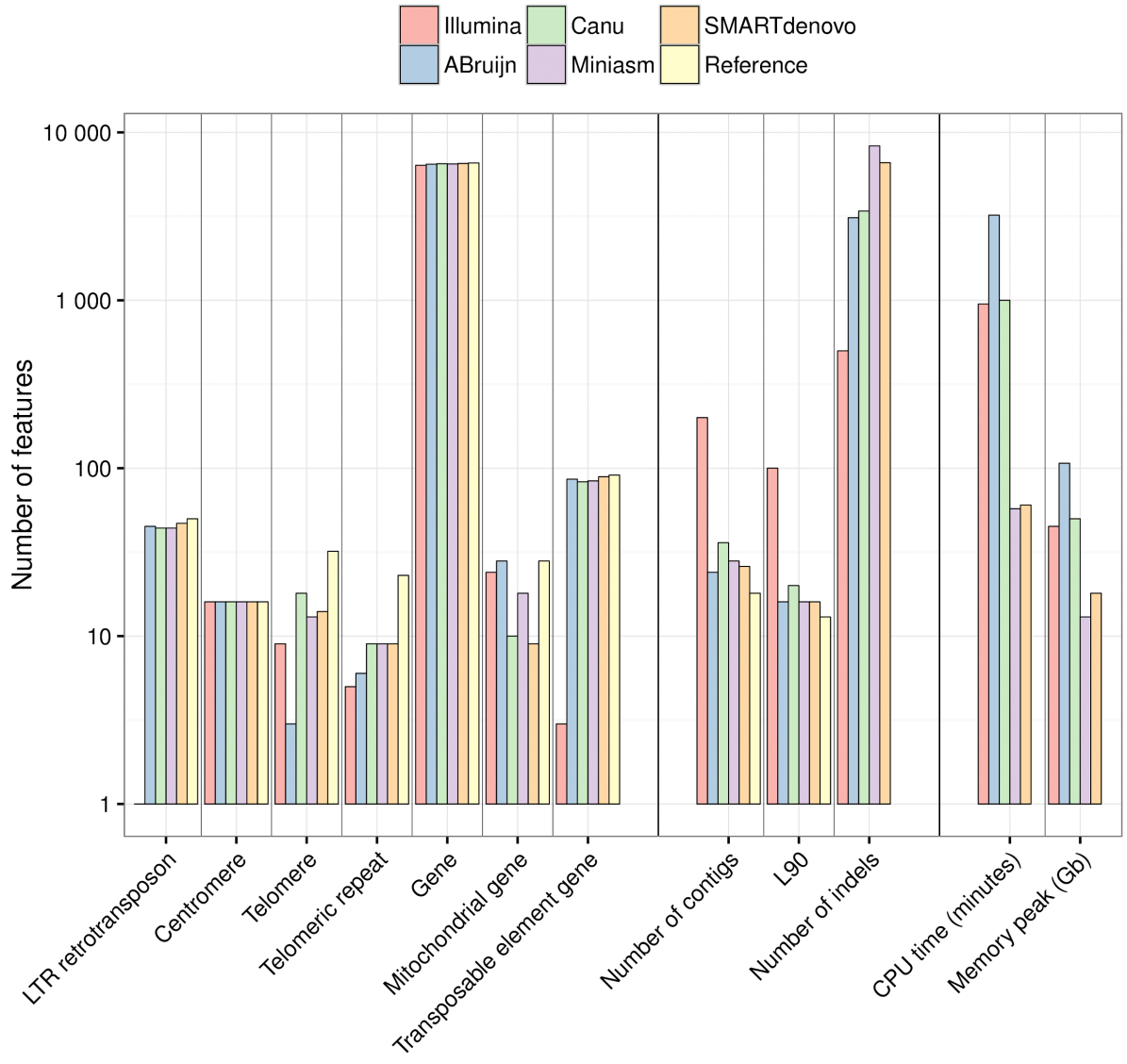

Figure 3: Cartography of the Ty transposon family. First and second tracks show, respectively, the percentage identity of the SMARTdenovo S288C assembly before and after polishing with Illumina paired-end reads using Pilon. The third track shows the 80th percentile number of contigs obtained for each strain and for all chromosomes. The remaining tracks show the density of Ty transposons or positions of the Ty1, Ty2, Ty3, Ty4, and Ty5 transposons across all the yeast strains. The red dot on the karyotype track shows the position of the rDNA cluster.

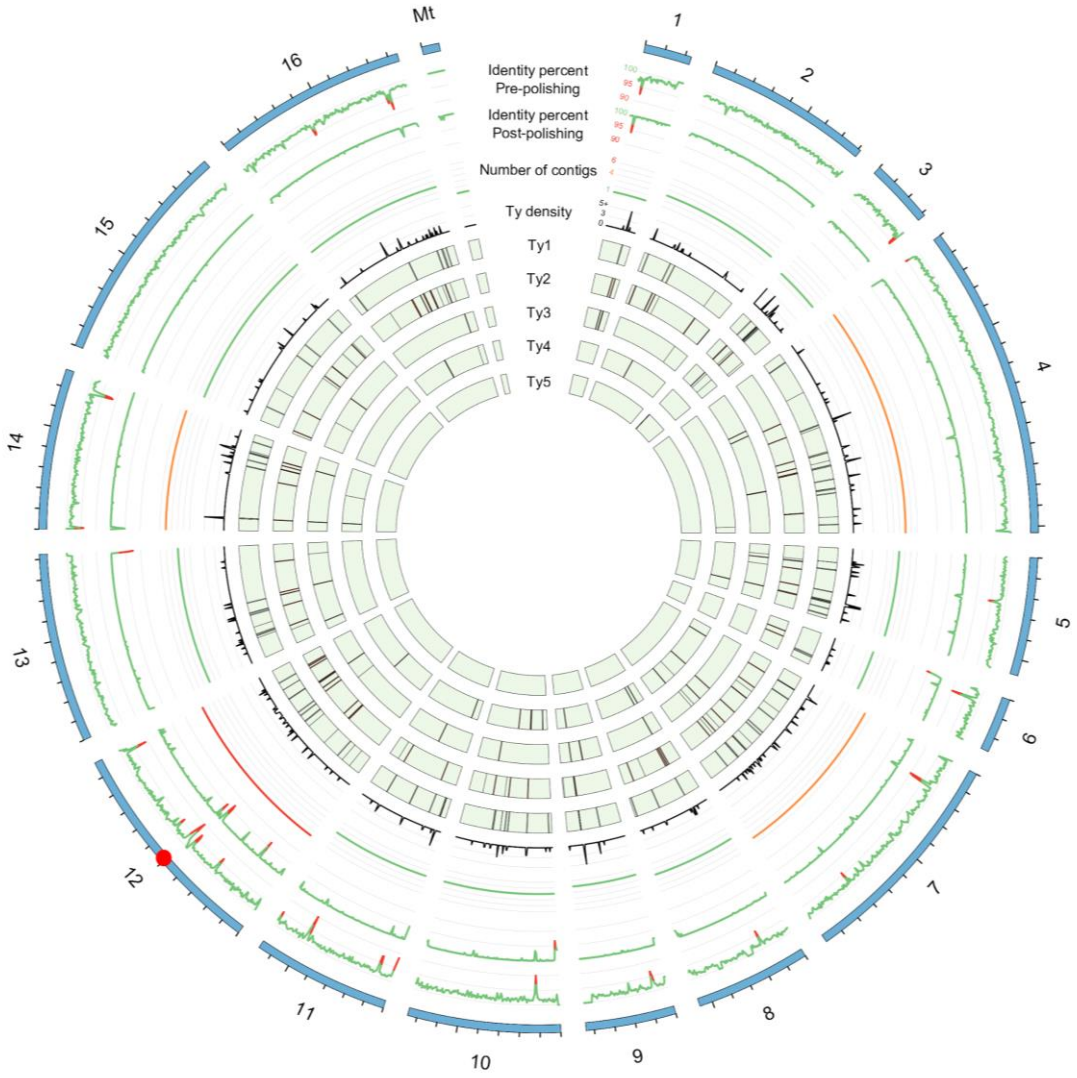

Table 1: Metrics of the SMARTdenovo S288C assemblies before and after polishing with Nanopolish using R7 reads. The Nanopore 2D reads were aligned to the most continuous SMARTdenovo assembly. The alignment was given as input to Nanopolish to correct assembly errors. Metrics were obtained by aligning the pre-polishing and post-polishing version of the assembly to the reference genome using Quast.

	SMARTdenovo Pre-polishing	**SMARTdenovo Post-polishing**
# contigs	26	26
Cumulative size	12,018,244	12,204,373
N50	771,149	782,423
N90	238,808	242,444
L50	7	7
L90	16	16
# mismatches	6,970	1,930
# insertions	7,735	7,707
# deletions	128,050	17,445
# deletions in homopolymers	79,152	6,869
# genes	6,251 + 24 partial	6,273 + 15 partial
# genes without indels	429	2,590

Table 2: Metrics of the S288C assemblies after polishing. Assemblies were corrected using 300x of 2x250bp Illumina reads as input to Pilon. The resulting corrected assembly was then aligned to the S288C reference genome using Quast.

	Spades	**Canu**	**Miniasm**	**SMARTdenovo**	**ABruijn**
Reads dataset used	Illumina PE 2x250 bp	2D pass	Canu-corrected	Longest 2D	2D
Coverage	300x	67x	108x	30x	120x
# reads > 10kb	0	16,860	21,005	28,668	28,668
# contigs	376	37	28	26	23
Cumulative size	12,047,788	12,230,747	12,113,521	12,213,590	12,182,847
Genome fraction (%)	96.464	98.519	98.421	99.352	98.635
N50	149,184	610,494	736,456	783,336	816,355
N90	19,522	191,846	265,917	242,658	257,117
L50	27	8	7	7	6
L90	100	20	16	16	16
# mismatches	1,126	1,898	4,455	4,205	2,138
# mismatches per 100 kb	9.47	15.85	37.23	34.27	17.88
# insertions	81	1,657	3,164	2,384	1,325
# deletions	439	1,869	5,208	5,551	1,838
# deletions in homopolymers	38	868	4,248	4,023	740
#indels per 100 kb	1.97	22.49	57.27	46.76	21.76
# genes	6,087 + 177 partial	6,241 + 32 partial	6,215 + 37 partial	6,266 + 33 partial	6,243 + 45 partial
# genes without indels	6,023	5,921	5,475	5,881	6,002

Table 3: Assembly metrics of the SMARTdenovo assemblies of all yeast strain genomes.

	# contigs	Cumul (bp)	N50 (bp)	N90 (bp)	L50	L90	Max size (bp)
ABH	22	11,960,929	803,880	267,734	6	16	1,483,918
ADM	41	11,883,044	474,542	171,488	10	26	1,009,064
ADQ	26	11,828,347	896,166	223,992	6	18	1,223,692
ADS	33	11,706,636	524,733	247,699	9	21	1,050,223
AEG	23	12,026,175	681,360	273,814	7	16	1,244,014
AKR	25	11,911,766	729,090	243,900	7	17	1,056,085
ANE	47	11,900,397	312,705	144,286	11	31	933,716
ASN	40	11,904,493	394,798	143,405	11	28	846,371
AVB	31	11,991,127	609,633	199,011	7	20	1,225,549
BAH	28	11,829,394	571,862	227,561	8	20	1,066,359
BAL	27	11,907,375	678,155	269,114	7	19	1,075,839
BAM	105	11,996,380	162,412	53,623	24	72	450,388
BCN	19	11,775,292	785,507	458,793	6	14	1,410,650
BDF	45	12,068,568	460,458	116,953	10	29	863,099
BHH	26	11,973,506	577,727	221,661	7	18	1,530,377
CBM	68	11,553,446	258,798	86,167	16	44	521,412
CEI	18	11,987,201	800,227	451,575	6	14	1,480,681
CFA	24	11,834,226	726,317	225,716	7	17	1,032,352
CFF	81	12,162,869	236,957	83,285	18	54	550,022
CIC	96	12,016,445	201,870	63,799	22	63	377,026
CNT	22	12,171,929	800,046	440,742	6	14	1,402,970
CRV (S288C)	26	12,213,584	783,337	242,658	7	16	1,532,642
Median	27.5	11,936,347	593,680	224,854	7	19.5	1,061,222
Reference	17	12,157,105	924,431	439,888	6	13	1,531,933

Table 4: Number of copies of multiple transposons across all yeast strains assemblies.

	Ty1	Ty2	Ty3	Ty4	Ty5
ABH	4	7	6	3	2
ADM	5	8	1	1	0
ADQ	4	7	1	2	0
ADS	1	9	0	0	1
AEG	15	7	2	1	2
AKR	4	4	4	1	1
ANE	1	5	3	2	0
ASN	13	6	0	0	0
AVB	0	29	0	0	2
BAH	0	6	1	3	0
BAL	8	0	12	0	0
BAM	4	13	6	2	1
BCN	6	0	0	0	0
BDF	13	3	3	3	1
BHH	20	12	5	4	0
CBM	3	1	0	1	0
CEI	2	20	1	0	0
CFA	8	1	1	0	1
CFF	6	6	2	0	1
CIC	6	3	1	1	0
CNT	17	6	1	1	1
CRV (S288C)	36	13	2	3	1
Reference	31	13	2	3	1

Table 5: Copy number of *CUP1* and *ENA1-2* tandem-repeated genes across the 21 natural isolates assemblies.

	ENA1-2	*CUP1*
ABH	1	10
ADM	2	1
ADQ	1	1
ADS	2	3
AEG	2	10
AKR	1	1
ANE	1	1
ASN	1	3
AVB	4	2
BAH	1	1
BAL	1	1
BAM	1	2
BCN	1	1
BDF	4	4
BHH	5	3
CBM	1	1
CEI	1	1
CFA	1	1
CFF	2	4
CIC	2	4
CNT	2	1

Table 6: Chromosomic rearrangements detected across all 21 strains.

Strain	Chromosome 1	Chromosome 2	Type
ABH	5	14	Translocation
ABH	5	14	Translocation
ABH	5	14	Translocation
ABH	14	14	Inversion
ADM	2	4	Translocation
ADM	5	7	Translocation
AKR	15	4	Translocation
ANE	16	5	Translocation
ANE	9	14	Translocation
ASN	5	2	Translocation
AVB	12	7	Translocation
AVB	7	12	Translocation
BAH	4	7	Translocation
BAH	10	9	Translocation
BAL	8	9	Translocation
BAM	4	7	Translocation
BAM	12	13	Translocation
BCN	6	13	Translocation
BCN	6	15	Translocation
BDF	4	14	Translocation
BDF	4	4	Inversion
BDF	5	12	Translocation
BDF	10	5	Translocation
BHH	12	12	Inversion
BHH	12	12	Inversion
CBM	16	3	Translocation
CBM	4	7	Translocation
CBM	12	15	Translocation
CEI	11	12	Translocation
CFF	14	12	Translocation
CIC	11	8	Translocation
CIC	4	7	Translocation
CNT	6	14	Translocation

Supplementary File1

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Supplementary Material

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Supplementary File2

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Supplementary Material

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Supplementary File3

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Supplementary Material

Supplementary_File3.xlsx

Author's covering letter for re-submission

Dr Laurie Goodman
Editor in Chief
GigaScience

15th November 2016

Dear Dr Goodman,

We now have addressed the reviewer comments and we hope that the manuscript is suitable for publication in Gigascience. All our changes in the main text of the article are underlined in yellow.

The manuscript has been reviewed and approved by all listed authors. All the datasets used in the article are available at: http://www.genoscope.cns.fr/yeast.

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

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Reviewer's report

Title: de novo assembly and population genomic survey of natural yeast isolates with the Oxford Nanopore MinION sequencer

Reviewer number: 1

Reviewer's report:

The authors describe assembly experiments on a set of yeast isolates using Oxford Nanopore MinION technology, both the older (and now discontinued) R7.3 and the newer (but about to be superceded) R9 chemistries. The methods are well-described, the data has been deposited in a stable archive and the results section performs a number of useful assessments of the quality of the assemblies using different de novo assembly tools.

Table S2 appears to be a subset of the columns of Table 1. If it doesn't provide any additional information, it shoudl be dropped.

Answer: Table S2 contains the metrics of the raw nanopore assemblies while Table 1 contains the metrics of the post-polished assemblies. We kept Table S2 in the supplementary data.

I would prefer that Table S4 be moved out of the supplement and into the main article. Details on polishing effects are important for understanding the ONT platform, and therefore it is unfortunate to bury them in the supplement. I might also argue that Tables S8 and S9 are unfortunate to maroon in the supplement, as these are demonstrating the value of the long read assembly.

Answer: We moved Tables S4, S8 and S9 in the main text.

Reviewer number: 2

Reviewer's report:

Authors present a survey on de novo assembly of yeast genomes using Oxford Nanopore MinION sequencer. Authors assembled a total of 22 yeast strains, the Saccharomyces cerevisiae S288C used to asses the quality and performances of the assemblers and data, and 21 strains selected for their diversity and spread.
They compare various types of data that can be produced with MiniION (e.g. 2D and 1D reads) and different MIniIon chemestries (R7.3 and the more recent R9).

Data is assembled using 4 different MiniIon only assemblers: Canu], Miniasm, SMARTdenovo and ABruijn.

They perform many assemblies with different types of data as input. They also use Illumina read to error correct the final assembly with Pilon.

In general I like the paper, it is a snapshot of the current status of de novo assembly with MiniIon and gives the possibility to a reader to have an idea of what tools to use and what results to expect.

I have some concerns that I want the authors to address:
- they often say in the text "kept the best assembly for each software" (e.g., page 6 line 141). They employ many metrics to discuss about assembly (contiguity, gene coverage, indels) and I like it a lot, but it is not clear how they select the "best" assembly. If for example they choose the best assembly based only on contiguity they might be constantly choosing assemblies affected by many errors, while less contiguous assemblies might be characterized by more correct sequences

Answer: We modified the text at several locations (lines 143,144,150,155,159 and 228) and we replaced "best assembly" by "most contiguous assembly". Indeed, we selected the best assembly for each method based on contiguity metrics (N50, Number of contigs and cumulative size).

- page 6 line 152: "a high proportion of mismateches and indels" : this needs to be more specific, what is "high proportion"?

Answer: We modified the corresponding sentence (lines 153-154), and replaced "a high proportion of mismatches and indels" by "the higher number of mismatches and indels". Furthermore we added a reference to Table S2 which contains the metrics (number of mismatches and indels) of the nanopore-only assemblies before the polishing step.

- The abstract is pretty positive about using only MiniIon data in de novo assembly, or at least that is my impression. Moreover, from the abstract and from the introduction part I was expecting to read about a MiniIon only evaluation and comparison. Instead, the assemblies presented in Table 1 and the various discussions on the evaluation show that all MiniIon assemblies needed Pilon (and therefore the 300X Illumina coverage) to be corrected. Moreover, to finish up the gneomes 8Kbp and 20Kbp library have been used, and I assume these are MP Illumina libraries. Therefore, I am now pretty skeptical about the ability of miniion to assemble alone yeast genome... I think that the abstract needs to be toned down and pint point more the need of complementary technologies to obtain a final assembly.

Answer: We take into account the reviewer comments and change the last sentence of the abstract (lines 56-59) to better reflect current issues with nanopore-only assemblies. All the assemblies were based on nanopore and Illumina paired-end sequencing; we didn't sequence any mate-pairs data. The nanopore-only assemblies show an accurate structure (organization of genomic elements, like genes or transposons) but the final consensus of those assemblies still remains problematic.

I want to point out this paper (I am one of the authors) https://gigascience.biomedcentral.com/articles/10.1186/s13742-015-0094-1
In this paper a multi-technology approch is followed combining Illumina, PacBio, and Optical Maps on an yeast genome. In case you have a similar variaty to the one assembled in this paper would be nice to compare the assembly presented in the paper with the MiniIon assembly... This is a plus, but I think it would really show potentials of MiniIon.

Answer: It would be very interesting to compare the results of a MinION sequencing strategy and the multi-technology approach (i.e. Illumina, PacBio and Optical Map), which was used to assemble the genome of the Dekkera bruxellensis yeast in Olsen et al. - GigaScience 2015. However, because we sequenced Saccharomyces cerevisiae genomes in our study, this is something impossible. Indeed, Dekkera bruxellensis and Saccharomyces cerevisiae are not closely related species and their genomes are very different in terms of chromosome number and there is no conservation of synteny at all.

Reviewer number: 3

Reviewer's report:

This is an excellent, timely, and well put together study. The results will be greatly helpful to many working on integrating this technology into the genome sequencing ecosystem.

Lines 195-196 describe read polishing with Pilon. It would be helpful to indicate what this depth of coverage was used to polish with the 2x250bp - I realize its in the table legend but could be helpful to include in the text here. also might be helpful to know if 300x is really needed to correct / polish well - would 100x work equally well?

Answer: We modified the text accordingly; we added the Illumina coverage that was used during the polishing step (line 198). Concerning the optimal coverage, we agreed with the reviewer and we performed several polishing with subsets of Illumina PE reads (from 25X to 300X). We added several sentences in the text (lines 205-209) and a supplementary figure (Figure S6) to show that a low coverage (100X or less) is sufficient to correct the consensus of nanopore-only assemblies.

Lines 209-216. Comparing the SPAdes Illumina assembly to the Nanopore only assembly -- The Table presents the QUAST(?) results that gene completeness is actually lower in the Illumina-only assembly but these are mostly indel free? Could be mentioned in the text here?

Answer: We added a sentence to underline these features (lines 214-216).

Doesn't SPAdes also have a option for co-assembly with Illumina + MinION data? Did this produce a useful / comparable assembly ?

Answer: That's right, but we'd like to focus our comparison on nanopore-only assemblers. However, we launched Spades with Illumina and nanopore data. The output assembly was composed of 143 contigs with a N50 near 250Kb. Although the assembly is less fragmented than the Illumina-only assembly it still remains highly fragmented compared to nanopore-only assemblies.

Lines 240 - 257. Sequencing the additional strains. It was unclear how the Pilon polishing is done here - the authors say 300x Illumina paired-end reads - are these from the same strain? Were illumina libraries made and sequenced for each strain or was this using the 1002 genome data ? (the 1002 site says it used 2x102 bp?)

Answer: We modified the text to better describe the pilon process (line 257) and we added a section in the method chapter to explain how the 22 genomes have been assembled (lines 504-509).

One idea I had in reading the manuscript. An additional type of repeat variation that is seen in Saccharomyces and other yeasts is the changes regarding simple repeats. These are particularly interesting in context when they fall within context of genic region generating instability that leads to phenotypic variation as the authors I am sure are aware. This was explored through PCR and sequencing in multiple strains by Verstrepen et al Nat Gen 2005 - in particular FLO1 has variable repeated regions easy to pick out. I searched FLO1 against the assemblies and found nice example of expanded repeat in the gene either matching the FLO5 or FLO1 copies. I worked up the example here
https://gist.github.com/hyphaltip/9f5256854f7a049ad81847c4740ece94#file-flo_loci-table
So it looks like there is variability in the size of the repeats in a few of these strains. Up to the authors if this is worth remarking on but it might be something that could also be better resolved than in Illumina assembly.

Answer: This is definitively an interesting comment. Indeed, there are repeated regions in the FLO1 gene, which additionally have an impact on the phenotypic diversity (e.g. adhesion, flocculation or biofilm formation). FLO1 is 4.6 kb long and contains a variable number of repeats of approx. 100 nt, separated by a 45-nt sequence. Consequently, these repeated structures having a small size can be resolved using Illumina sequencing data. In the frame of our study, we really wanted to focus on larger repeated structures, i.e. involving entire genes such as ENA and CUP genes tandem arrays. Indeed, long read sequencing technologies should have a high resolution compared to short read strategies.

Excellent description of methods, versions of software used, and providing reproducible methods. Though it changes rarely, it may be useful to spell out the exact version of the S288C genome assembly and GFF files used in validation.

Answer: We'd like to thank the reviewer for its conscientious reading of the manuscript as well as its suggestions of improvements.