Oxford NANOPORE Technologies

MICROBIOLOGY

CASE STUDY

High-quality, low-cost, nanopore-only bacterial genome sequences

To obtain reference-quality bacterial genome assemblies, data is often used from the sequencing of either pure cultures or metagenomic samples. Short-read sequencing has been the technology of choice for this application in previous years but has limited ability to resolve repetitive sequences that are longer than the library insert size. Consequently, technology capable of producing long sequencing reads, including the Oxford Nanopore platform, has '*recently emerged as the choice*' for assembling genomes derived from such samples[1].

Professor Albertson and colleagues, based at Aalborg University in Denmark, investigated whether nanopore sequencing data alone could be used to obtain reference-quality bacterial genome assemblies[1]. Their work noted that, in the past, there has been a preference to use either short-read or reference polishing of nanopore data to obtain near-complete microbial genome assemblies, yet this is an undesirable option as it adds cost and complexity[1].

The team evaluated the performance of R9 and the more recent R10 nanopore chemistry in bacterial genome assembly, obtaining sequence data derived from 'pure cultures' (in this case, a mock community) and an activated sludge sample.

They introduced the term 'near-finished' genome to indicate the generation of a high-quality genome assembled with only long nanopore reads, for which the application of short-read polishing would not significantly improve the consensus sequence. They found that R10.4 data alone could generate near-finished bacterial genomes, without polishing (**Figure 1**). The depth of coverage required to achieve this was approximately 40-fold. To assess performance on metagenomic genome assembly, the team sequenced a sample of activated sludge; a similar conclusion was made — that R10.4 chemistry enabled the generation of near-finished microbial genomes, without short-read polishing[1].

Products used

Kit	Ligation Sequencing Kit
Devices	MinION™ | PromethION™
Tools	Flye | minimap2 | Racon | Medka

Find out more: nanoporetech.com/products

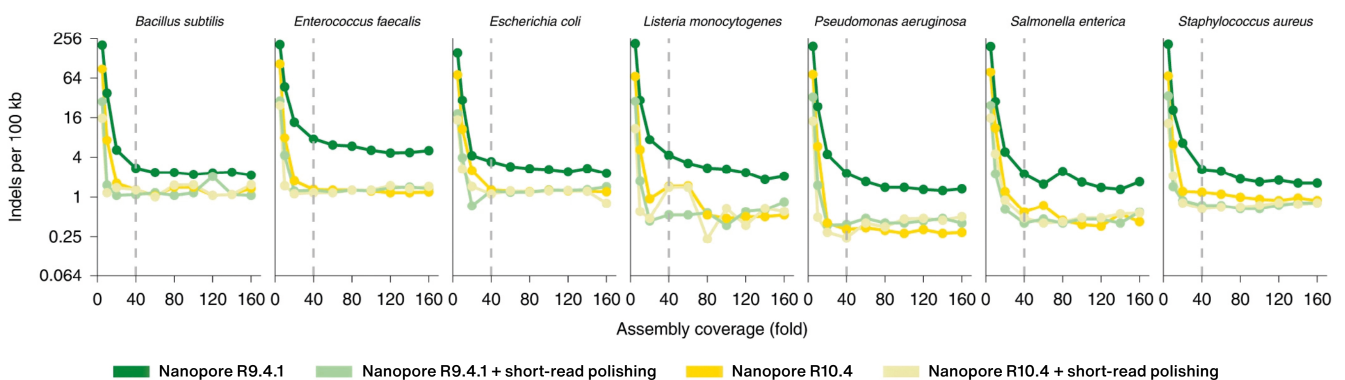

Figure 1
Indels observed per 100 kb in the *de novo* bacterial isolate assemblies, at different depths of coverage, with and without short-read polishing. The authors noted that short-read polishing of nanopore data obtained using R10.4 chemistry provided no significant improvement in assembly quality. Image adapted from Sereika *et al.*[1] and available under Creative Commons license (creativecommons.org/licenses/by/4.0).

The pathogen *Mycobacterium tuberculosis* is responsible for tuberculosis (TB), which remains one of the deadliest infectious diseases, with 1.5 million human deaths attributed to TB in 2020[2]. Drug-resistant *M. tuberculosis* is a particularly significant threat for effective TB control[2,3].

Previously, short-read sequencing technology was typically used to investigate the genetic basis of resistance and the genomics underpinning TB transmission. However, the genome of *M. tuberculosis* is challenging to resolve with short reads due to its high GC content and repetitive nature — including the highly variable and GC-rich *pe/ppe* genes associated with drug resistance, which are often excluded from analysis due to difficulties in accurately mapping these regions to the genome when using short reads. Furthermore, the high capital cost and centralisation associated with these sequencing platforms has limited access to whole-genome analysis in many areas with a high TB burden and lower income[3].

In contrast, the Oxford Nanopore platform can produce sequencing reads of any length, and a scalable range of devices is available, including portable options suitable for *in situ* sequencing; the technology has therefore been recognised as a '*promising platform for cost-effective application*' to TB genome analysis[3].

In light of this, Gómez-González *et al.* sequenced 10 *M. tuberculosis* clinical research isolates with both nanopore and short-read technology, obtaining 93.6-fold short-read and 72.2-fold nanopore depth of coverage, after mapping[3]. The team highlighted the improved coverage of long nanopore reads in repetitive regions where short reads failed to accurately align. As expected, a higher number of large variants were detected with long nanopore reads (median 81 vs. 24, across the isolates). In addition, for all sample pairs, 99% of single nucleotide polymorphisms (SNPs) were called in both samples, with few platform discrepancies. All lineage predictions were identical between the two platforms; however, looking specifically at the nanopore data, as the *pe/ppe* gene regions were successfully resolved with long nanopore reads, SNPs could also be incorporated from these regions for lineage analysis, which led to an improved resolution that '*would be of special interest in outbreak settings, where transmission analysis of closely related isolates can be potentially better established*'. They also suggested that the ability to cover repetitive regions with long reads could contribute a better understanding of drug-resistance mechanisms in *M. tuberculosis*[3].

Products used

Kit	Ligation Sequencing Kit
Device	MinION
Tools	Bonito | BEDTools | Freebayes | Delly | TB-profiler

Find out more: nanoporetech.com/products

NANOPORE SEQUENCING

- enabled reference-quality genome assembly without short-read polishing
- resolved repetitive and GC-rich regions intractable to short-read sequencing technologies

"Oxford Nanopore R10.4 enables the generation of near-finished microbial genomes from pure cultures or metagenomes at coverages of 40-fold without short-read polishing[1]"

Find out more about microbial sequencing using nanopore technology: nanoporetech.com/applications/microbiology

References

1. Sereika, M. et al. Nat. Methods. 19, 823–826 (2022).
2. WHO. Tuberculosis. Available at: https://www.who.int/news-room/fact-sheets/detail/tuberculosis [Accessed: 23 August 2022]
3. Gómez-González, P.J. et al. Briefings in Bioinformatics. bbac256, https://doi.org/10.1093/bib/bbac256 (2022).

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