MICROBIAL WHOLE GENOME SEQUENCING — BEST PRACTICES

Make reference-quality genomes your new standard practice with highly accurate long reads (HiFi reads) and our end-to-end workflow on the Sequel® IIe system.

- Generate closed chromosomes and plasmids from even the most repeat-dense and GC-rich genomes easily, affordably, and at high throughput
- Identify ever-evolving genes associated with toxicity, virulence, and antimicrobial resistance
- Precisely identify strains, serotypes, and plasmids to track pathogen outbreaks in human, plant, and animal species, through food systems, hospitals, and communities
- Comprehensively characterize microbes to facilitate scientific breakthroughs and discovery

From genomic DNA to complete genome in a single experiment

Workflow recommendations

- Start with the recommended input of high-quality DNA (1.0 μg)
- Shear DNA to 7–10 kb to obtain fragment lengths optimal for HiFi sequencing
- Multiplex up to 96 samples, or up to 375 Mb of total genome per SMRT® Cell 8M, to assemble most bacterial chromosomes into single contigs
- Use our microbial multiplexing calculator to simplify equimolar pooling
- Use SMRT® Link v10.2 for fully automated de-multiplexing, assembly, circularization, and polishing of both chromosomes and plasmids to produce gold standard references
- Achieve high-quality consensus accuracies >99.99%
- Output data in standard file formats (BAM and FASTA/Q) for seamless integration with downstream analysis tools
Generate microbial reference genomes by multiplexing up to 96 isolates on the Sequel II system

Sequence the whole story with closed genomes and plasmids

PacBio sequencing of E. coli found on retail meats allowed scientists at National Antimicrobial Resistance Monitoring System (NARMS) to fully characterize plasmid-mediated quinolone resistance (PMQR) genes. The study revealed novel plasmid backbones and evidence of co-selection of quinolone resistance and resistance to antimicrobials commonly used in animal feed and to treat severe infections in humans. The authors note, "These details are important in assessing the nature of resistant microbial hazards in food and other sources." 4

Microbial assembly statistics from a 96-plex pool of bacteria relevant to food safety and human health. These data were generated on the Sequel II system and assembled with the fully automated HiFi-based Microbial Assembly application in SMRT Link v10.2 using the default parameters, without any manual curation. Download and explore the data yourself. 4

Accuracy of representative samples from the above 96-plex sequencing run. With HiFi data and the Microbial Assembly application in SMRT Link v10.2, genome assemblies are consistently >99.99% accurate.

KEY REFERENCES
3. SMRT Link user guide (v10.2). PacBio documentation.
4. November 2021 Microbial 96plex Dataset.