### MICROBIAL WHOLE GENOME SEQUENCING

# Make reference-quality genomes your new standard practice with highly accurate long reads—HiFi reads—and the PacBio® end-to-end workflow



Generate closed chromosomes and plasmids from even the most repeat-dense and GCrich genomes easily, affordably, and at high throughput



Precisely identify strains, serotypes, and plasmids to help track pathogen outbreaks in human, plant, and animal species, through food systems, hospitals, and communities

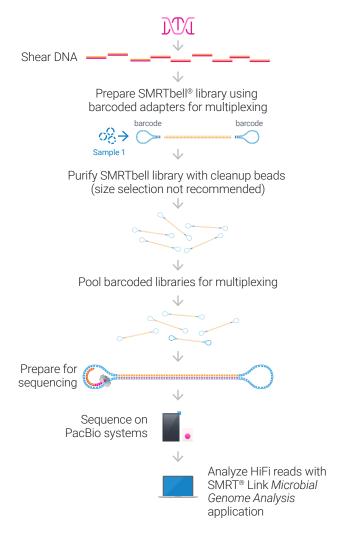


**Identify ever-evolving genes** associated with toxicity, virulence, and antimicrobial resistance



More comprehensively characterize microbes to facilitate scientific breakthroughs and discovery

## From genomic DNA to a complete genome in a single experiment



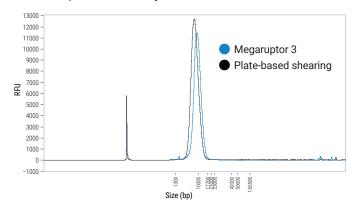
#### Workflow recommendations

- Start with the recommended input of high-quality DNA (50-300 ng)<sup>1,2</sup>
- Shear DNA to ~10-15 kb with low- or highthroughput methods for flexible batch sizes to obtain fragment lengths optimal for HiFi sequencing<sup>3</sup>
- Use the HiFi plex prep kit 96<sup>1</sup> to multiplex up to 384 samples, or up to 2 Gb of total genome per SMRT<sup>®</sup> Cell to assemble most bacterial chromosomes into single contigs, with accessory plasmids, for <\$50/ sample\*</li>
- Use SMRT Link for fully automated demultiplexing, assembly, circularization, and polishing of both chromosomes and plasmids to produce gold standard references<sup>4</sup> and optionally include analysis of 6mA and 4mC modified bases and associated DNA sequence motifs
- Achieve high-quality consensus accuracies >99.99%
- Output data in standard file formats (BAM and FASTA/Q) for seamless integration with downstream analysis tools
- Identify strain characteristics, AMR genes, and mobile vectors mediating spread

\*Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Vega™ or Revio® system and does not include instrument amortization, other reagents, or DNA extraction.

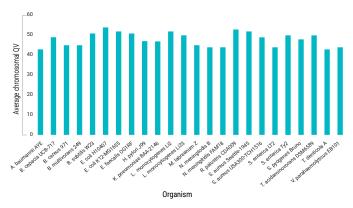


### High-throughput shearing methods yield consistent and comparable library sizes



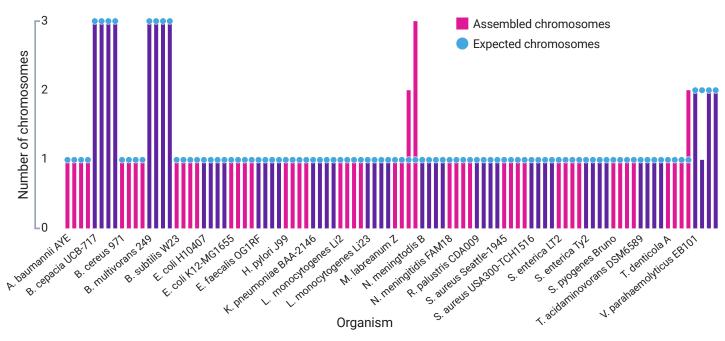
SMRTbell library size distributions for *E. coli* gDNA sheared on the *Megaruptor 3* system and with plate-based shearing. The *Megaruptor 3* sheared DNA (blue) and the plate-sheared DNA (black) yielded similar size distributions with modal sizes of 8.4 kb and 9.3 kb, respectively. DNA sizing was performed on the *Femto Pulse* system.<sup>3</sup>

### HiFi assemblies are highly accurate



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

### Generate microbial reference genomes at scale



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.<sup>5</sup>



#### Learn more about microbial whole genome sequencing: pacb.com/microbial-wgs

- 1. Procedure & checklist Preparing multiplexed whole genome and amplicon libraries using the HiFi plex prep kit 96
- Overview HiFi application options and sequencing recommendations
- 3. Technical note High-throughput DNA shearing for long-read microbial WGS
- 4. Documentation SMRT Link user guide
- 5. Dataset Microbial 96plex dataset

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