

## 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 GC-rich 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

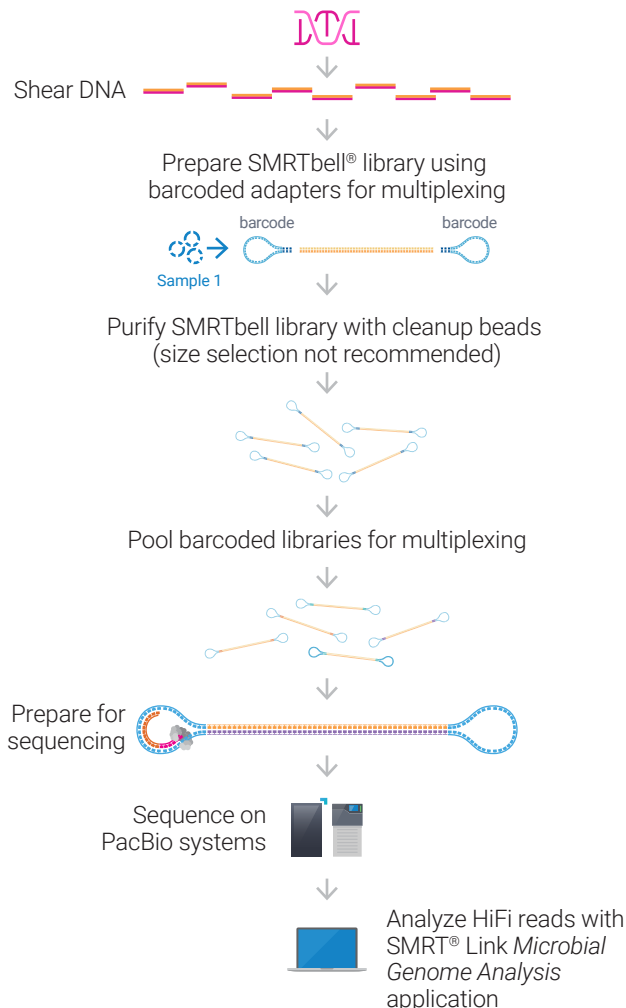


**Identify ever-evolving genes** associated with toxicity, virulence, and antimicrobial resistance



**More comprehensively characterize microbes** to facilitate scientific breakthroughs and discovery

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

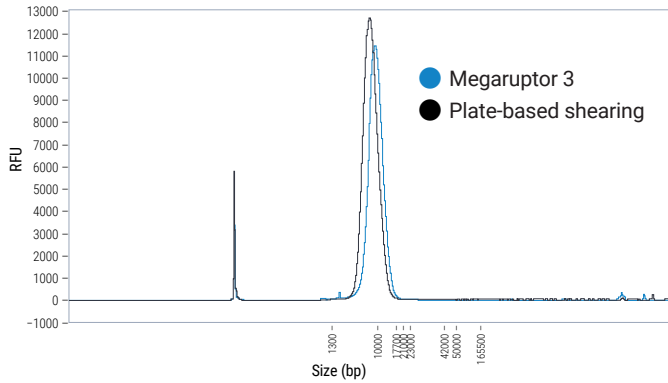


### Workflow recommendations

- 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 high-throughput 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 375 Mb or 1.2 Gb of total genome per SMRT® Cell 8M or 25M, respectively, to assemble most bacterial chromosomes into single contigs, with accessory plasmids, for <\$50/sample\*
- 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

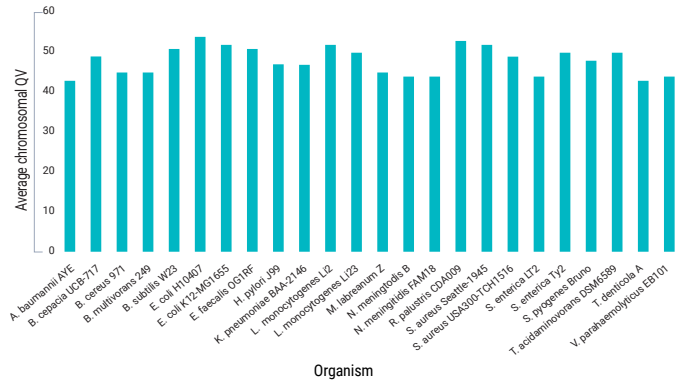
\*Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel® II/III or Revio™ system and does not include instrument amortization, other reagents, or DNA extraction.

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



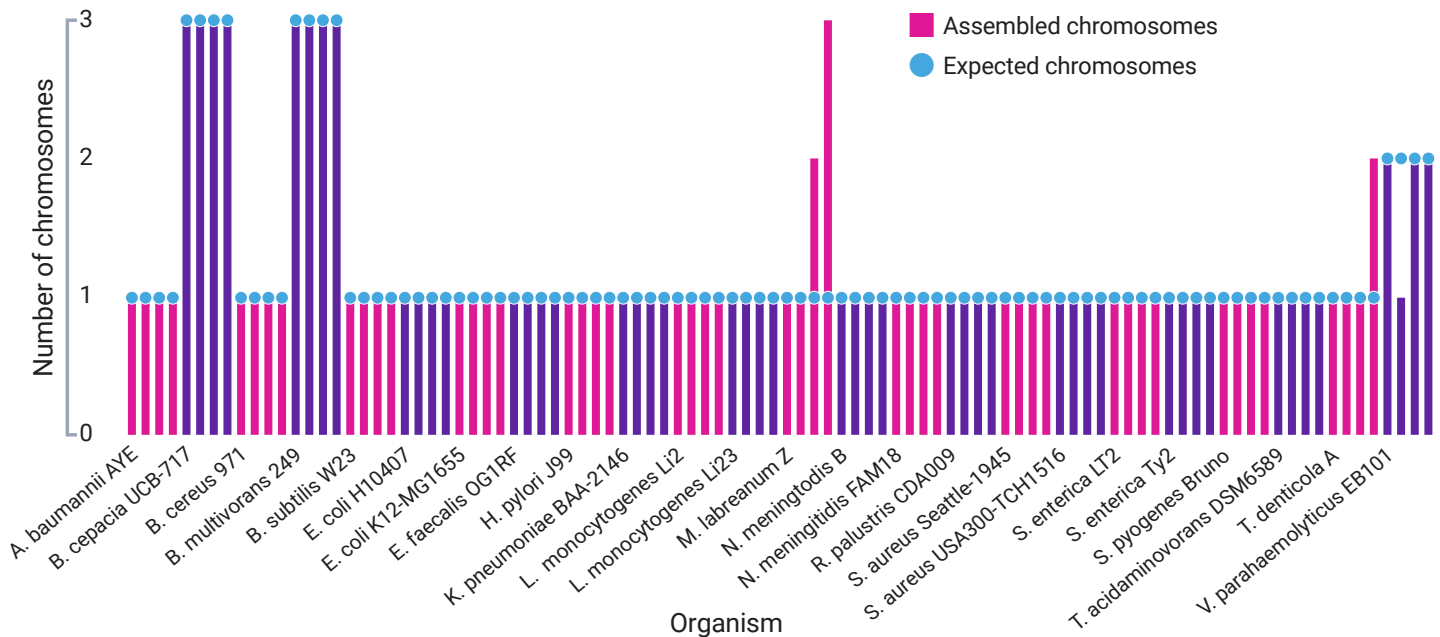
SMRTbell library size distributions for *E. coli* gDNA sheared on the *Megaruptor 3* system and with plate-based shearing. The *Megaruptor 3* sheared DNA (blue) and the plate-sheared DNA (black) yielded similar size distributions with modal sizes of 8.4 kb and 9.3 kb, respectively. DNA sizing was performed 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 *Microbial Assembly* application in SMRT Link v10.2 and later, genome assemblies are consistently >99.99% accurate.

## Generate microbial reference genomes by multiplexing up to 96 isolates



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](https://pacb.com/microbial-wgs)

1. Procedure & checklist – Preparing multiplexed whole genome and amplicon libraries using the HiFi plex prep kit 96
2. 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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