Application brief

MICROBIAL WHOLE GENOME SEQUENCING

Make reference-quality genomes your new standard practice with highly accurate long reads (HiFi reads) and our end-to-end workflow

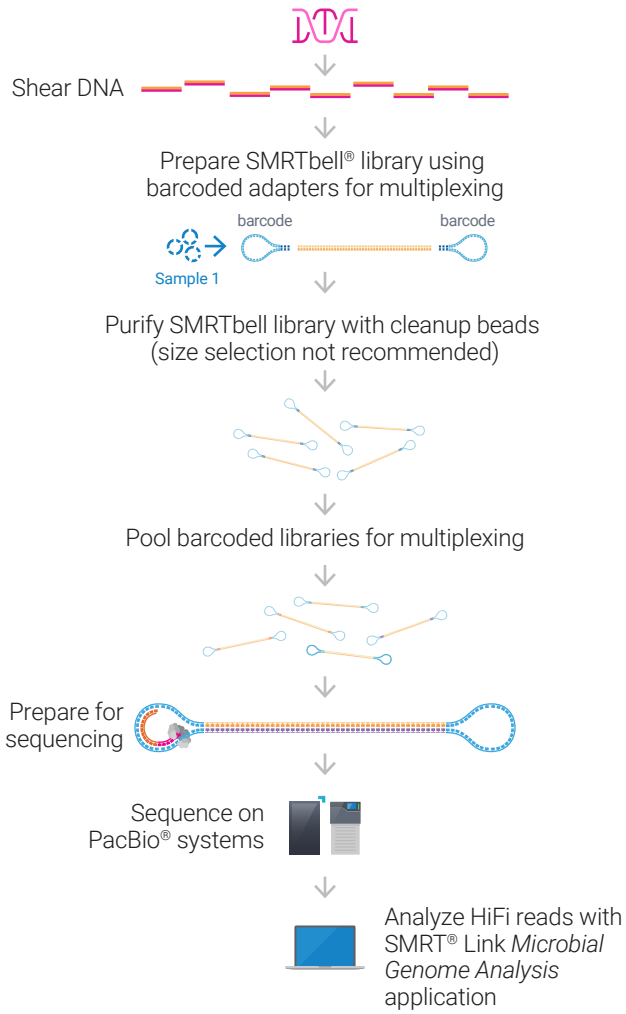
Generate closed chromosomes and plasmids from even the most repeat-dense and GC-rich genomes easily, affordably, and at high throughput

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

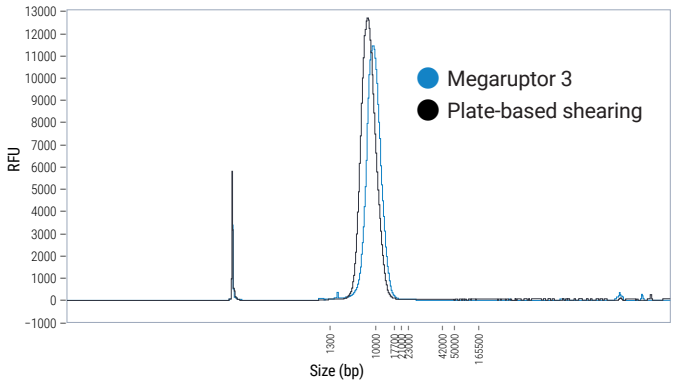
From genomic DNA to a complete genome in a single experiment



Workflow recommendations

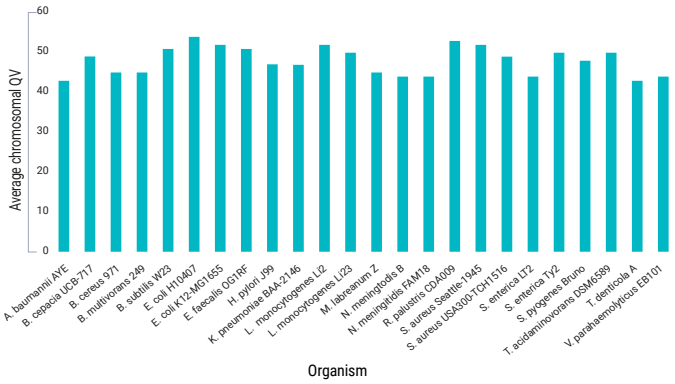
- Start with the recommended input of high-quality DNA (300 ng)[1,2]
- Shear DNA to 7–10 kb with low- or high-throughput methods for flexible batch sizes to obtain fragment lengths optimal for HiFi sequencing[3]
- Multiplex up to 96 samples, or up to 375 Mb or 1.2 Gb of total genome per SMRT® Cell 8M or 25M, respectively, to assemble most bacterial chromosomes into single contigs
- Use SMRT Link for fully automated demultiplexing, assembly, circularization, and polishing of both chromosomes and plasmids to produce gold standard references[4] 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

High-throughput shearing methods yield consistent and comparable library sizes



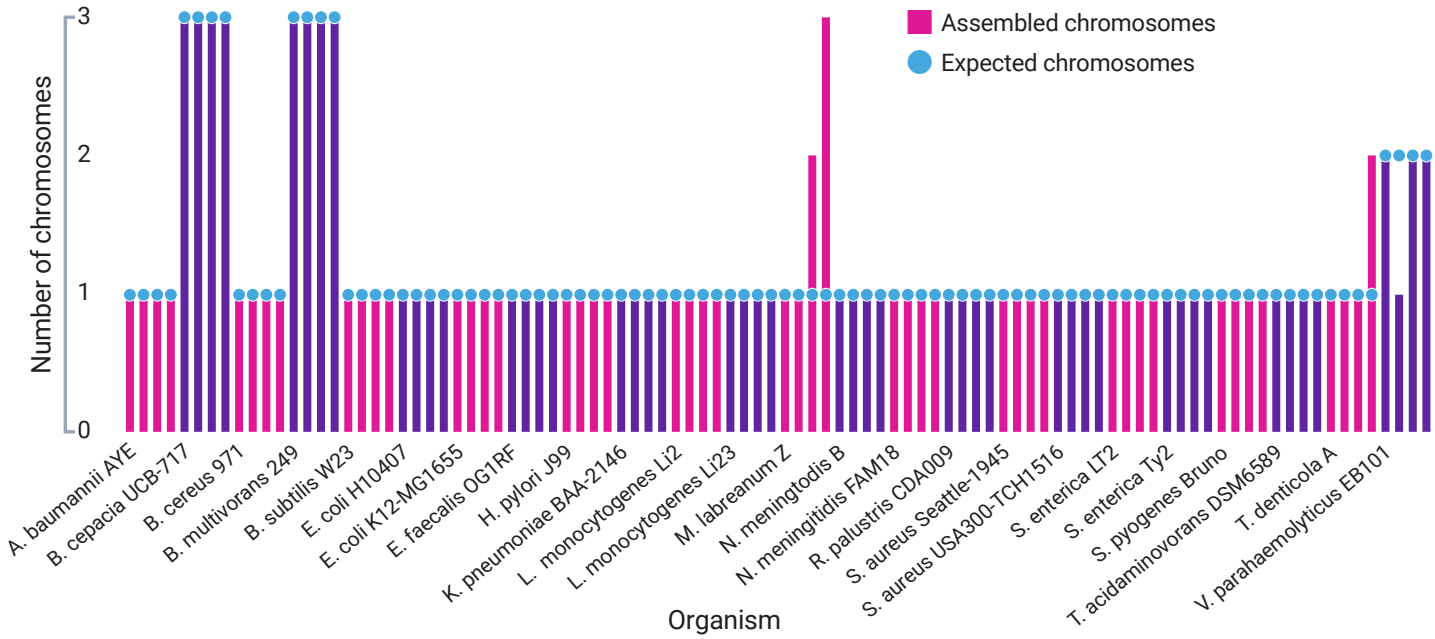
SMRTbell library size distributions for *E. coli* gDNA sheared on the *Megaruptor 3* system and with plate-based shearing. The *Megaruptor 3* sheared DNA (blue) and the plate-sheared DNA (black) yielded similar size distributions with modal sizes of 8.4 kb and 9.3 kb, respectively. DNA sizing was performed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

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.[5]

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

1. Procedure & checklist — Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0
2. Overview — Sequel systems 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