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 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.

Comprehensively characterize microbes to facilitate scientific breakthroughs and discovery.

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

- Shear DNA
- Prepare SMRTbell® library using barcoded adapters for multiplexing
- Purify SMRTbell library with cleanup beads (size selection not recommended)
- Pool barcoded libraries for multiplexing
- Prepare for sequencing
- Sequence on PacBio® systems
- Analyze HiFi reads with SMRT Link Microbial Genome Analysis application

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 sequencing3
- 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 for fully automated de-multiplexing, assembly, circularization, and polishing of both chromosomes and plasmids to produce gold standard references4
- Achieve high-quality consensus accuracies >99.99%
- Output data in standard file formats (BAM and FASTA/Q) for seamless integration with downstream analysis tools
High-throughput shearing methods yield consistent and comparable library sizes

![Graph showing 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.]

HiFi assemblies are highly accurate

![Graph showing 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

![Graph showing assembled chromosomes and expected chromosomes for various organisms.]

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.

Learn more about microbial whole genome sequencing: [pacb.com/microbial-wgs](https://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

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