MICROBIAL WHOLE GENOME SEQUENCING
BEST PRACTICES

With Single Molecule, Real-Time (SMRT®) Sequencing, you can affordably characterize complete microbial genomes. For most microbes, closed genomes and accessory plasmids can be assembled using PacBio® data from single libraries in a single run.

FROM GENOMIC DNA TO COMPLETE GENOME IN A SINGLE EXPERIMENT

SAMPLE PREPARATION RECOMMENDATIONS
- Use our barcoded adaptor kits to reduce the cost of microbial multiplexing1
- Multiplex up to 30 Mb of microbial genomes per SMRT Cell 1M on the Sequel® System to assemble most bacterial genomes into 5 contigs or fewer, exclusive of plasmids
  - Use our Microbial Multiplexing Calculator to simplify equimolar pooling2
  - Adjust planned multiplexing depth to balance cost constraints with your requirements for genome completeness2
- Closure of class III complexity genomes with large repeat regions may require 20–30 kb library preparations and may not be suitable for multiplexing2,3
- Use the recommended starting input of high-quality DNA (1.0 μg)

GENERATE NEAR-COMPLETE MICROBIAL ASSEMBLIES WITH MULTIPLEXING ON THE SEQUEL SYSTEMS

Shown above are Hierarchical Genome Assembly Process (HGAP) de novo assembly statistics from a pool of bacteria relevant to food safety and nosocomial infections, sequenced in one attempt using the standard protocol and assembled using the recommended HGAP parameters without adjustment. These data were generated on the Sequel System, using SMRTbell® Template Prep Express Kit 2.0, and 3.0 Chemistry.
DATA ANALYSIS SOLUTIONS WITH SMRT ANALYSIS AND OUR PARTNERS

- Easily generate gold-standard genome and plasmid de novo assemblies with automated de-multiplexing of pooled samples and one-size-fits-most HGAP4 settings through the SMRT Analysis user interface4,5
- Alternatively, use cloud-based implementations of SMRT Analysis available from our data analysis partners
- Achieve high-quality consensus accuracies >99.999%
- Output data in standard file formats, BAM and FASTA/Q, for seamless integration with downstream analysis tools
- Annotate active m6A and m4C R-M system motifs with the ‘Base Modification and Motif Analysis’ workflow in SMRT Analysis 6.0.

HIERARCHICAL GENOME ASSEMBLY PROCESS

CHARACTERIZE COMPLETE MICROBIAL GENOMES AND METHYLOMES

1. Procedure & Checklist - Preparing Multiplexed Microbial Libraries Using SMRTbell Express Template Prep Kit 2.0.
2. Application Note: Microbial Multiplexing Workflow on the Sequel System.
4. Tutorial: Multiplexed Microbial Assembly.
5. Analysis Procedure – Multiplexed Microbial Assembly with SMRT Link v6.0.0 and SMRTbell Express Template Prep Kit 2.0

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