

METAGENOMIC SEQUENCING WITH HIFI READS

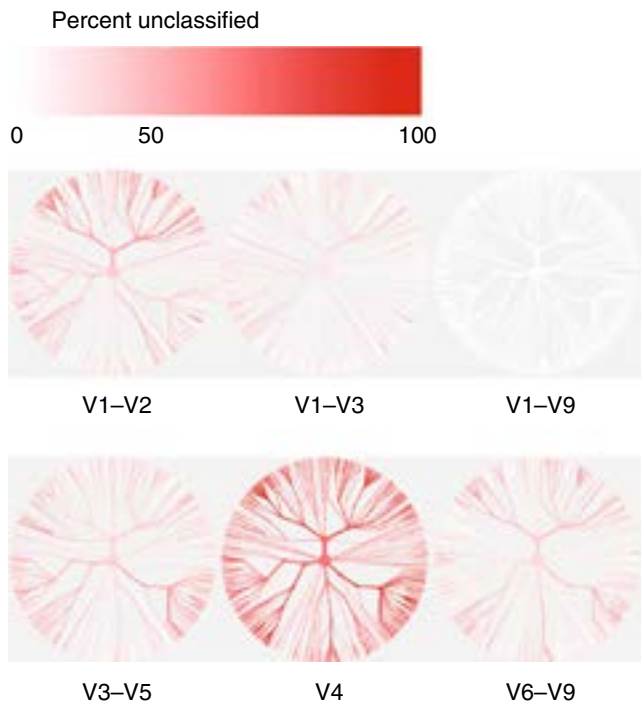
BEST PRACTICES



Highly accurate long reads – HiFi reads – with single-molecule resolution make Single Molecule, Real-Time (SMRT®) Sequencing ideal for full-length 16S rRNA sequencing, shotgun metagenomic profiling, and metagenome assembly.

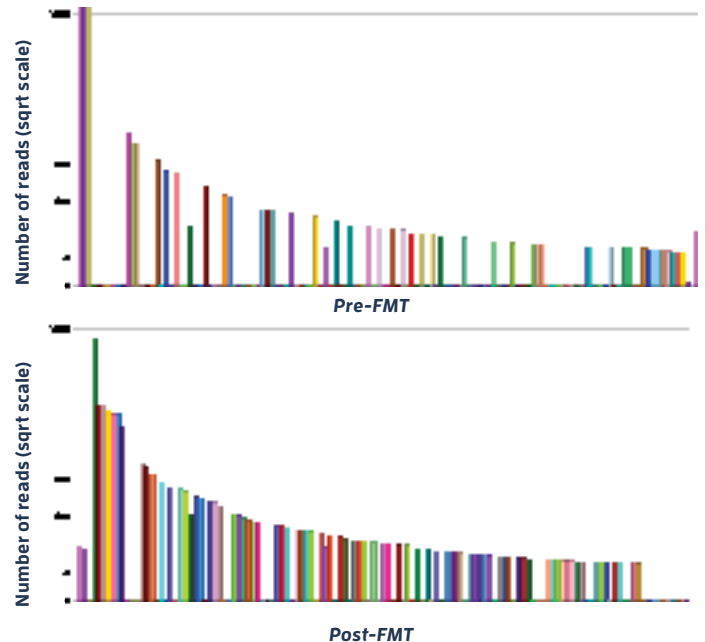
- Identify 6-8 full-length genes in every HiFi read, without assembly, with cost-effective metagenomic profiling
- Determine community composition at the species or strain-level with affordable full-length 16S sequencing
- Generate reference-quality assemblies with 15-fold coverage of HiFi metagenome data for comparative genomics
- Leverage epigenomic data to associate contigs and plasmids from closely related strains

FULL-LENGTH 16S rRNA SEQUENCING: SPECIES-LEVEL PHYLOGENETIC RESOLUTION



The proportion of 16S sequences from each bacterial genus that cannot be identified at the species level varies significantly depending which variable region is used. Since the human gut can harbor a broad diversity of bacterial clades, only full length sequences (V1-V9) can provide unbiased resolution of all the species that may be present¹.

LONG-READ METAGENOMIC PROFILING: FECAL MICROBIOME DIVERSITY



Long, single-molecule reads generated high-resolution metagenomic profiles down to the species and strain level for pre- and post-fecal-microbiota-transplant (FMT) samples from an individual suffering from chronic *C. difficile* infection².

FROM DNA TO RESOLVED MICROBIAL COMMUNITIES



| | Full-length 16S rRNA Sequencing | Shotgun Metagenomic Profiling | Metagenome Assembly |
|--|---|--|---|
| | High-resolution, cost-effective screening for microbial community studies | Unbiased discovery and functional characterization of microbial communities | Generation of complete or near-complete genome assemblies from microbial population |
| Library Preparation³ | | | |
| Prepare SMRTbell[®] Template | Full-length 16S rRNA protocol ⁴ available with recommended full-length primers or use Shoreline Biome ⁶ kits for DNA extraction and PCR amplification | Create 10 kb libraries for HiFi metagenomics ⁵ | |
| | Multiplexing and library automation supported | | |
| SMRT Sequencing with the Sequel[®] II System | | | |
| Yield | 1.6 kb HiFi reads | 10 kb HiFi reads | |
| | A 6-hour movie collection generates: Up to 3.5 million >Q20 reads | A 30-hour movie collection generates: Up to 2.4 million >Q20 reads | |
| Scale Throughput* | Resolve up to 96 communities per SMRT Cell 8M for \$15/sample [†] | Profile 3 communities per SMRT Cell 8M for \$450/sample [†] Recommended coverage: 5-fold coverage of least abundant species for intact genes and operons | Generate assemblies of high-complexity samples for \$1300/sample [†] Recommended coverage: 15-fold coverage for reference-quality genome assemblies |
| | Data Analysis Tools Available Through SMRT Analysis or PacBio[®] Devnet | | |
| SMRT Analysis | Circular consensus sequencing (CCS) analysis produces HiFi reads through multiple observations of single circularized templates | | <i>De novo</i> assemble high-quality metagenomes to high consensus accuracies >99.999% with Canu ⁷ |
| Tertiary Analysis | Analyze 16S data with either Shoreline Biome SB Analyzer ⁴ or DADA2 ⁸ | Study metagenomic profile and functions using public databases and MEGAN ⁹ | Utilize epigenetic signatures to improve shotgun metagenomic analysis ¹⁰ |

* Read lengths, reads/data per SMRT Cell 8M and other sequencing performance results vary based on sample quality/type and insert size.

† Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II System and does not include instrument amortization or other reagents.

KEY REFERENCES

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- Hall, R., et al. (Feb, 2017) Using the PacBio Sequel System to taxonomically and functionally classify metagenomic samples in a trial of patients undergoing fecal microbiota transplantation. *Poster presented at Advances in Genome Biology and Technology*. Hollywood, Florida.
- Overview - Sequel Systems application options and sequencing recommendations. PacBio Documentation.
- Procedure & Checklist - Full-length 16S amplification, SMRTbell library preparation and sequencing. PacBio Documentation.
- Procedure & Checklist - Preparing 10 kb Library Using SMRTbell Express Template Prep Kit 2.0 for Metagenomics Shotgun Sequencing. PacBio Documentation.
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