

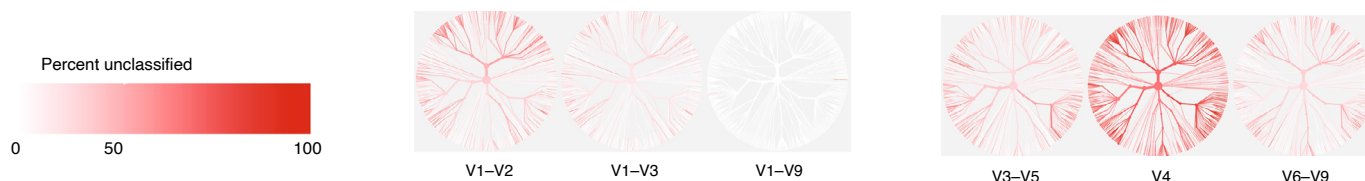
MICROBIOME AND METAGENOME SEQUENCING WITH HIFI READS

Highly accurate long reads – HiFi reads – are the new benchmark for full-length 16S, metagenome profiling, and metagenome assembly

- Determine community composition at the species or strain level with competitively priced full-length 16S sequencing.
- Profile metagenomes efficiently and cost-effectively with an average of eight full-length genes in every HiFi read.
- Generate hundreds of high-quality (HQ) metagenome assembled genomes (MAGs), many of which are circular single contigs.
- Leverage epigenomic data to associate contigs and plasmids from closely related strains.

Full-length 16S rRNA sequencing

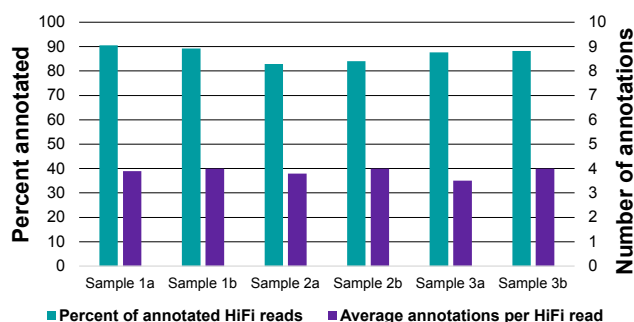
Achieve species- and strain-level phylogenetic resolution with the Kinnex™ 16S rRNA kit¹ at a highly competitive cost relative to short-read partial 16S sequencing.



The proportion of 16S sequences from each bacterial genus that cannot be identified at the species level varies significantly depending on 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 present.²

HiFi metagenomics

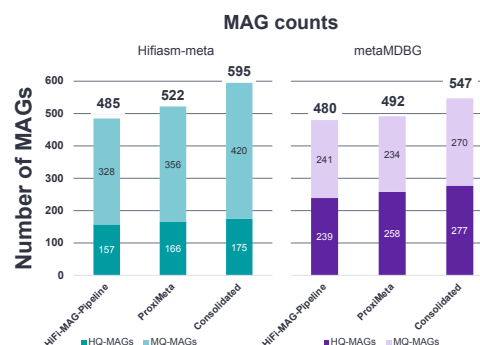
Generate precise species characterization, more functional annotations, more HQ MAGs, and more circular MAGs, even at lower coverage. HiFi metagenomes generated with the HiFi plex prep kit 96³ or HiFi prep kit 96⁴ are cost competitive with short-read metagenomes.



With up to nine complete genes per HiFi read, PacBio® data provides rich functional information; nearly every read contributes to your understanding of the biological functions present in your microbial community.⁵

Obtain more and richer metagenome functional information

- ~80–90% of HiFi reads are functionally annotatable
- Each HiFi read typically has an average of four functional annotations



The unique combination of long read lengths and high accuracy overcomes many challenges involved with metagenome assembly such as distinguishing closely related strains and yielding single-contig, high-quality MAGs. PacBio HiFi sequencing of the ZymoBIOMICS human fecal reference. For binning, the HiFi-MAG-Pipeline, Phase Genomics ProxiMeta, and a consolidated set of the two was used, which further increases the number of MAGs recovered.⁶

Achieve standout metagenome assemblies

- Across all method combinations: 595 total MAGs, 277 HQ-MAGs, and 98 single-contig HQ-MAGs (including 70 circular)
- These MAGs represent up to 455 species clusters, with 15% of the MAGs representing uncultured species (including >35 HQ-MAGs)⁶

From DNA* to resolved microbial communities

Samples + reads/data per sample per SMRT® Cell† and cost estimate‡							
Protocol/library prep ⁷			Vega™ system		Revio® system with SPRQ™ chemistry		Analysis tools
Full-length 16S rRNA High-resolution, cost-effective screening for microbial community studies	Amplification of bacterial full-length 16S gene with barcoded primers ⁸	Preparing Kinnex™ libraries from 16S rRNA amplicons ⁹	Standard 16S 384 samples ~10k reads ~\$5	Kinnex 16S 1,152 samples ~30k reads ~\$4	Standard 16S 384 samples ~20k reads ~\$5	Kinnex 16S 1,536 samples ~45k reads ~\$4	Analyze full-length 16S data with either DADA2, ¹² QIIME 2, ¹³ microbiomehelper, ¹⁴ OneCodex, ¹⁵ EZBiome, ¹⁶ or with the PacBio GitHub pipeline ¹⁷
Metagenome profiling Unbiased compositional and functional characterization of microbial communities	Create ~10–15 kb libraries for HiFi metagenome sequencing Preparing multiplexed whole genome and amplicon libraries using the HiFi plex prep kit 96 ³ , the Ampli-Fi protocol ¹⁰ , or the seqWell LongPlex kit ¹¹		64 samples ~0.75 Gb ~\$57 ³ ~\$63 ¹⁰ ~\$44 ¹¹		128 samples ~0.75 Gb ~\$47 ³ ~\$53 ¹⁰ ~\$34 ¹¹		Study metagenome taxa and functions using <i>DIAMOND</i> and MEGAN-LR ¹⁸ or Sourmash ¹⁹ with the PacBio GitHub pipeline ²⁰ or with BugSeq ²¹
Metagenome assembly Generation of complete or near-complete MAGs from microbial populations	Create ~10–15 kb libraries for HiFi metagenome sequencing Preparing whole genome libraries using the HiFi prep kit 96 ⁴ , the Ampli-Fi protocol ¹⁰ , or the seqWell LongPlex kit ¹¹		8 samples ~7 Gb ~\$194 ⁴ ~\$189 ¹⁰ ~\$172 ¹¹		16 samples ~6 Gb ~\$119 ⁴ ~\$113 ¹⁰ ~\$92 ¹¹		<i>De novo</i> assemble high-quality metagenomes with hifiiasm-meta, ²² metaFlye, ²³ or metaMDBG ²⁴ and perform QC, binning, classification, and evaluation with the PacBio pipeline ²⁰

* Metagenomic DNA can be extracted using commercially available kits from suppliers such as Zymo Research and Qiagen. For metagenome sequencing libraries from sample types that may contain inhibitors or contaminants, it is recommended to further clean up the extracted DNA with a column-based cleanup such as the DNeasy PowerClean Pro Cleanup Kit (cat no. 12997-50).

† Read lengths, reads/data, and number of samples per SMRT Cell and other sequencing performance results may 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 Vega or Revio system and does not include instrument amortization, other reagents, or DNA extraction.

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7. Overview – HiFi application options and sequencing recommendations.

8. Procedure & checklist – Amplification of bacterial full-length 16S gene with barcoded primers.

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Learn about metagenome sequencing:

pacb.com/microbial-pop

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