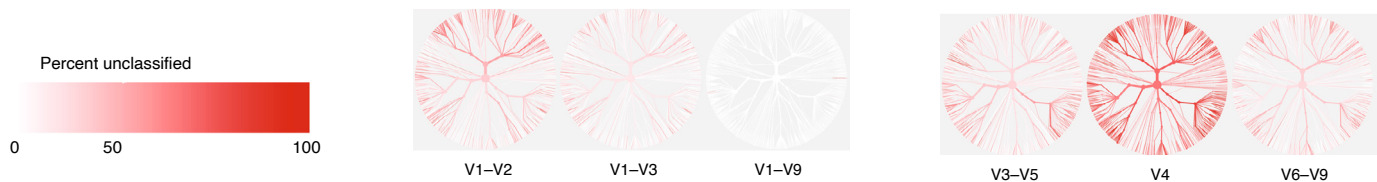


Highly accurate long reads – HiFi reads – are the new benchmark for full-length 16S, metagenome profiling, and metagenome assembly

- 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

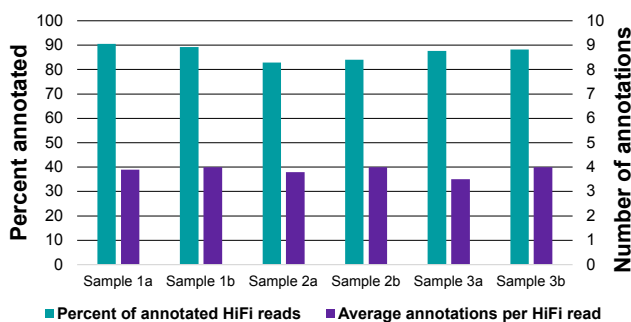
Achieve species- and strain-level phylogenetic resolution with the Kinnex™ 16S rRNA kit¹ at a highly competitive cost relative to short-read partial 16S sequencing.



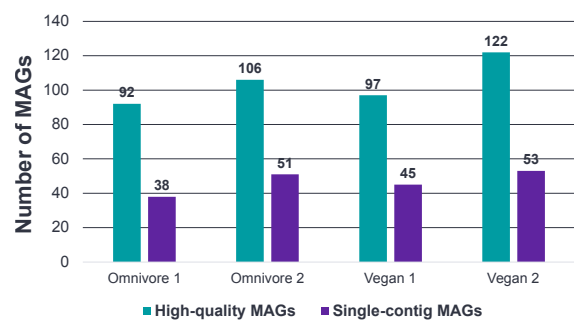
The proportion of 16S sequences from each bacterial genus that cannot be identified at the species level varies significantly depending on which variable region is used. Since the human gut can harbor a broad diversity of bacterial clades, only full-length sequences (V1–V9) can provide unbiased resolution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



The unique combination of long read lengths and high accuracy overcomes many challenges involved with metagenome assembly such as distinguishing closely related strains in the same sample and yielding single-contig MAGs. Analysis of four human gut microbiome samples from *The BioCollective*⁶ shows HQ MAGs ≥70% completeness, <10% contamination, <10 contigs.

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

Achieve standout metagenome assemblies

- ~90–125 HQ MAGs per sample with ~17 Gb data; many are single-contig with ~50 per sample
- 417 HQ MAGs in total across four samples

From DNA* to resolved microbial communities

Samples + reads/data per SMRT® Cell[†] and cost estimate[‡]

	Protocol/library prep ⁷		Sequel® II/Ile SMRT Cell 8M		Revio™ SMRT Cell 25M		Analysis tools
			Standard 16S	Kinnex 16S	Standard 16S	Kinnex 16S	
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	192 samples ~10k reads/sample ~\$9/sample	768 samples ~30k reads/sample ~\$5/sample	384 samples ~10k reads/sample ~\$5/sample	1,536 samples ~30k reads/sample ~\$4/sample	Analyze full-length 16S data with either DADA2, ¹⁰ QIIME 2, ¹¹ microbiomehelper, ¹² OneCodex, ¹³ EZBiome, ¹⁴ or with the PacBio GitHub pipeline ¹⁵
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		48 samples ~0.5 Gb/sample ~\$65/sample		96 samples ~0.75 Gb/sample ~\$49/sample		Study metagenome taxa and functions using <i>DIAMOND</i> and MEGAN-LR ¹⁶ or Sourmash ¹⁷ with the PacBio GitHub pipeline ¹⁸ or with BugSeq ¹⁹
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		4 samples ~6 Gb/sample ~\$385/sample		12 samples ~6 Gb/sample ~139/sample		<i>De novo</i> assemble high-quality metagenomes with hifiasm-meta, ²⁰ metaFlye, ²¹ or metaMDBG ²² and perform QC, binning, classification, and evaluation with the PacBio pipeline ¹⁸

* 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 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/Ile or Revio system and does not include instrument amortization, other reagents, or DNA extraction.

KEY REFERENCES

- Application note – Kinnex 16S rRNA kit for full-length 16S sequencing
- Johnson, J. S., et al. (2019) Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*. 10(1), 5029.
- Procedure & checklist – Preparing multiplexed whole genome and amplicon libraries using the HiFi plex prep kit 96
- Procedure & checklist – Preparing whole genome libraries using the HiFi prep kit 96
- Gehrig, J., et al. (2022) Finding the right fit: evaluation of short-read and long-read sequencing approaches to maximize the utility of clinical microbiome data. *Microbial Genomics*, 8(3), 10.1099/mgen.0.000794.
- <https://github.com/PacificBiosciences/pb-metagenomics-tools/blob/master/docs/PacBio-Data.md>
- Overview – HiFi application options and sequencing recommendations.
- Procedure & checklist – Amplification of bacterial full-length 16S gene with barcoded primers.
- Procedure & checklist – Preparing Kinnex libraries from 16S rRNA amplicons
- Callahan, B. J., et al. (2019) High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. *Nucleic Acids Research*, 47(18), e103.
- <https://qiime2.org>
- [https://github.com/LangilleLab/microbiome_helper/wiki/PacBio-CCS-Amplicon-SOP-v1-\(qiime2\)](https://github.com/LangilleLab/microbiome_helper/wiki/PacBio-CCS-Amplicon-SOP-v1-(qiime2))
- <https://onecodex.com>
- <https://ezbiome.com>
- <https://github.com/PacificBiosciences/HiFi-16S-workflow>
- Huson, D. H., et al. (2018) MEGAN-LR: new algorithms allow accurate binning and easy interactive exploration of metagenomic long reads and contigs. *Biology Direct*, 13(1), 6.
- Pierce, N. T., et al. (2019) Large-scale sequence comparisons with sourmash. *F1000Research*, 8, 1006.
- <https://github.com/PacificBiosciences/pb-metagenomics-tools>
- <https://bugseq.com> and <https://bugseq.com/pacbio>
- Feng, X., et al. (2022) Metagenome assembly of high-fidelity long reads with hifiasm-meta. *Nature Methods*, 10.1038/s41592-022-01478-3
- Kolmogorov, M., et al. (2020) metaFlye: scalable long-read metagenome assembly using repeat graphs. *Nature Methods*, 17(11), 1103–1110.
- Benoit, G., et al. (2024) High-quality metagenome assembly from long accurate reads with metaMDBG. *Nature Biotechnology*, 10.1038/s41587-023-01983-6.



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