Methods for 16S rRNA sequencing

Taxonomic profiling of bacterial communities and microbiomes with Illumina NGS solutions

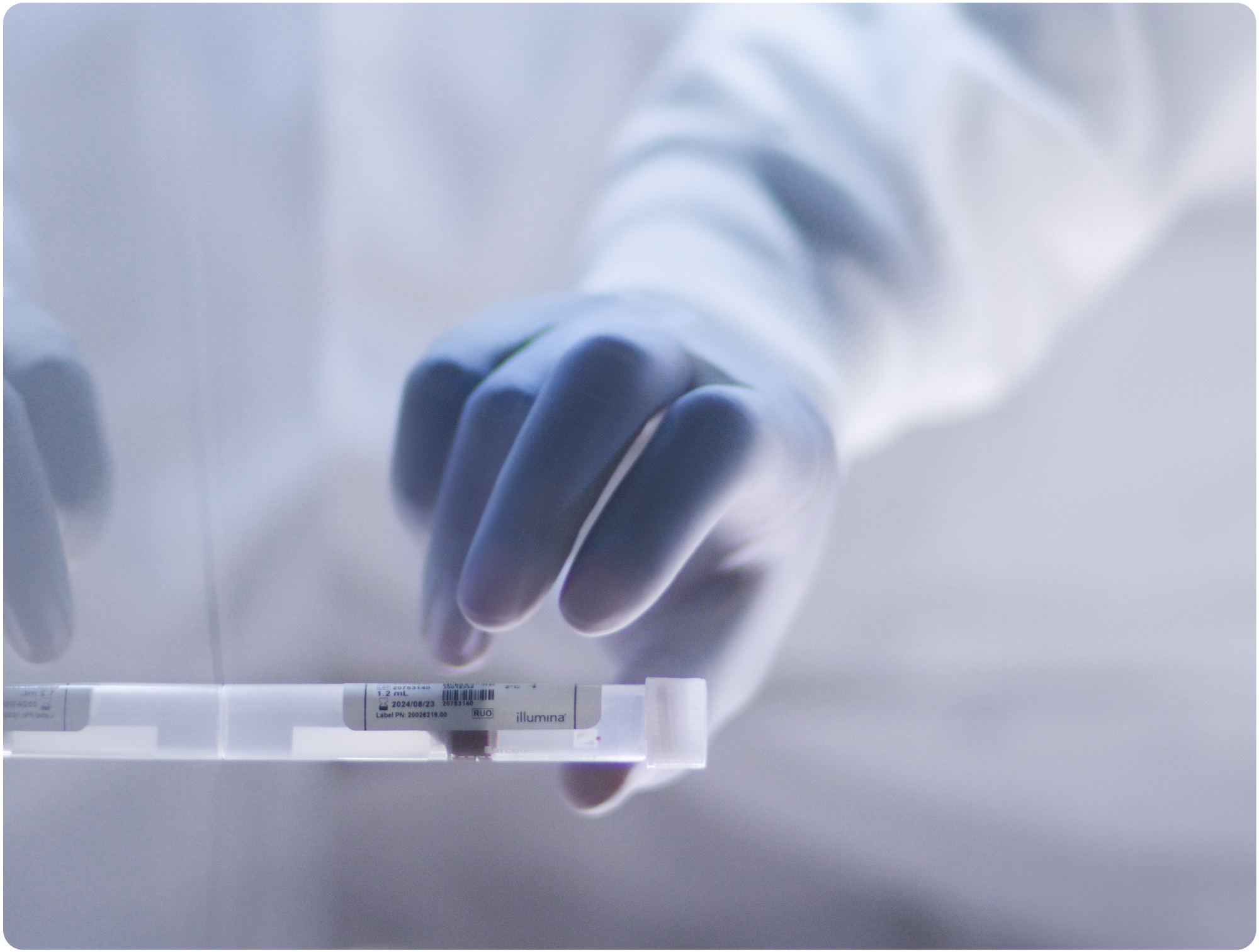
1.2 mL
2024/08/23
Label PN: 20026219.00
RUO
illumina

Table of contents

01 Introduction

In microbiology, the 16S ribosomal RNA (16S rRNA) gene is a single genetic locus, ~1500 bp long, containing nine variable regions interspersed between conserved regions. The 16S locus acts like a 'barcode' for differentiating microbial taxa. It can be used to classify bacteria and archaea taxonomically from within a heterogenous community to assess the diversity within a population and compare relative abundance across similar samples.

Using amplicon-based next-generation sequencing (NGS), researchers have compiled comprehensive databases for comparing 16S rRNA sequences throughout an ecosystem, including complex environments such as the human gut microbiome.[1,2]

Why choose 16S amplicon NGS for microbiome diversity studies?

Amplicon sequencing is a highly targeted approach that enables researchers to analyze genetic variation in specific genomic regions. In this method, PCR is used to amplify a portion of the 16S gene from many bacteria simultaneously. The amplified regions are then interrogated through DNA sequencing.

Comparing 16S rRNA gene sequencing to shotgun metagenomics

16S rRNA sequencing differs from shotgun metagenomics. Whereas 16S rRNA sequencing offers a taxonomic survey on the diversity of a single gene, shotgun metagenomics functionally characterizes the genomes of entire microbial communities.

16S rRNA sequencing is used to identify bacteria and archaea in complex samples and to estimate relative abundance within a sample. Drawing conclusions across different sample types is not recommended due to potential differences in total abundance.

Benefits of using NGS for 16S metagenomics:

- Simultaneous analysis of hundreds of thousands of different 16S amplicons from a single sample
- Targeted approach specific to bacteria/archaea can provide species-level identification
- Culture-free method to identify bacteria taxa that may not be detected using traditional methods
- Fast, high-throughput workflow enables rapid identification of bacterial composition within a microbiome and comparisons across samples

Shotgun metagenomics is used to analyze primary samples of all microbes, including viruses, fungi, and bacteria and provide a more comprehensive functional representationof the sample. Additionally, it can be used to determine bacterial taxonomy at the strain level, whereas 16S rRNA sequencing provides species-level taxonomy.

02 Applications for 16S rRNA sequencing

16S rRNA sequencing powers human and environmental microbiome studies.

Characterizing the complexity of the human microbiome

16S rRNA sequencing can be used to study the impacts the microbiome has on human health and disease. The Integrative Human Microbiome Project (HMP) uses 16S rRNA and other sequencing methods to study the microbiome at specific body sites. Researchers query and retrieve metagenomic, metatranscriptomic, human genetic, microbial culture and other data to increase their understanding of the microbiome.[3]

Bacterial identification of culture-negative infectious agents

For select scenarios, using 16S rRNA sequencing in culture-negative infections has improved bacterial pathogen identification. A retrospective study performed at the University Hospitals-Rainbow Babies and Children's Hospital determined 16S rRNA sequencing had potential clinical utility in diagnosing culture-negative infections.[4]

Tracking bacterial community structures in industrial wastewater

Industrial wastewater can adversely impact the microbial ecological environment, possibly resulting in pathogenic outbreaks and other threats at the community level. Wastewater surveillance can detect, identify, and characterize pathogens present in wastewater and inform public health responses. Researchers at the National Research Centre in Egypt used 16S rRNA sequencing to assess bacterial diversity from textile wastewater and environmental water samples. They found that microbial communities in textile wastewaters are similar to communities found in environmental samples at the phylum level but distinct at the genus and species levels because they are exposed to a wider range of environmental circumstances.[5]

03 16S rRNA sequencing: step-by-step overview

After DNA extraction, 16S rRNA sequencing has three basic steps: library preparation, sequencing, and analysis (Figure 1). During library preparation, Illumina sequencing adapters and dual-index barcodes are added to the 16S amplicon target. Prepared libraries are loaded onto a sequencing system and the resulting data are analyzed for taxonomic classification and relative abundance estimation.

Figure 1: 16S rRNA sequencing workflow.

STEP 1

Library preparation

Illumina does not offer library preparation kits specific for 16S rRNA sequencing; however, Illumina systems are compatible with third-party 16S rRNA sequencing library preparation kits. Illumina provides a protocol for preparing libraries for sequencing the variable V3 and V4 regions of the 16S rRNA gene.[6]

Parameter	16S sequencing protocol[a]
Input DNA quantity	10–15 ng
Assay time	~ 7 hr
Multiplexing capacity	384[b]
Genome coverage	V3 and V4 regions of the 16S rRNA gene; additional targets available depending on primer design
Sequencing method	Amplicon sequencing

a. Refer to the demonstrated protocol for 16S metagenomic sequencing library preparation.[6]
b. Multiplexing capacity is determined by the number of indexes available and instrument capacity.

STEP 2

Sequencing

Illumina NGS systems deliver exceptional data quality and accuracy with flexible throughput and simple, streamlined workflows. The iSeq™ 100 System and MiSeq™ i100 Series are ideally suited to perform 16S rRNA sequencing studies.

System	iSeq 100 System	MiSeq i100 Series[a]			
Flow cell	Reagent v2	5M	25M	50M	100M
Reads per flow cell	4M	5M	25M	50M	100M
Reads per sample	0.1M	0.1M			
No. of samples per flow cell[b]	38	47	237	384[c]	384[c]
Recommended read length	2 × 150 bp	2 × 300 bp			

a. MiSeq i100 supports 5M and 25M flow cells only. MiSeq i100 Plus System supports all listed flow cells.
b. Based on 5% PhiX.
c. Based on available Illumina indexes.

STEP 3

Data analysis

16S rRNA sequencing data can be seamlessly transferred, analyzed, and stored securely in BaseSpace™ Sequence Hub, the easy-to-use Illumina cloud-based genomic computing environment. The 16S Metagenomics app provides taxonomic composition, relative abundance, HTML visualizations (eg, sunburst charts and stacked bar charts), and an operational taxonomic unit (OTU) table that can be used for further statistical analyses.

Analysis application	Description	Input
16S Metagenomics	16S taxonomic classification and relative abundance for microbiome diversity studies	16S rRNA amplified DNA (FASTQ format)

04 Support that never stops

Illumina strives to be the best partner possible. With a global presence, you can rely on our support to facilitate your success. Technical support is available via phone five days a week or access online support 24/7, worldwide and in multiple languages, with rapid response time near most major metropolitan areas. Illumina provides excellent product consistency, supply, and quality enabled by a mature global manufacturing infrastructure.

Trusted technology, trusted partner

As a preferred NGS platform provider, Illumina has shipped over 20,000 sequencing systems globally. Illumina NGS technology is cited in over 421,000 peer-reviewed publications—5× more than all other NGS technologies combined.[7] Building on decades of expertise, Illumina has a relentless commitment to innovation and building future NGS capabilities and applications.

Illumina 16S rRNA sequencing

05 Ordering information

Library preparation

Product	No. of indexes[a]	No. of samples	Catalog no.
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)	96	96	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes, 96 Samples)	96	96	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 Indexes, 96 Samples)	96	96	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 Indexes, 96 Samples)	96	96	20091660
PhiX Control Kit, v3	—	—	FC-110-3001
Illumina Purification Beads, 30 ml	—	—	20119944

a. 384 indexes total available when combining sets A, B, C, and D.

Sequencing system

System	Catalog no.
iSeq 100 System	20021532
MiSeq i100 System	Coming Soon
MiSeq i100 Plus System	20115695

Data analysis

Product	Catalog no.
BaseSpace Sequence Hub Professional Annual Subscription[a]	20042109
BaseSpace Sequence Hub Enterprise Annual Subscription[a]	15066411

a. BaseSpace Sequence Hub subscriptions include complimentary iCredits for running analysis apps and data storage. Additional iCredits are available for purchase.

06 Appendix

References

1. McDonald D, Hyde E, Debelius JW, et al. American Gut: an Open Platform for Citizen Science Microbiome Research. *mSystems*. 2018;3(3):e00031-18. Published 2018 May 15. doi:10.1128/mSystems.00031-18.
2. McDonald D, Price MN, Goodrich J, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J*. 2012;6(3):610-618. doi:10.1038/ismej.2011.139.
3. Integrative HMP (iHMP) Research Network Consortium. The Integrative Human Microbiome Project. *Nature*. 2019;569(7758):641-648. doi:10.1038/s41586-019-1238-8
4. Lim PPC, Stempak LM, Malay S, Moore LN, Cherian SSS, Desai AP. Determining the Clinical Utility of 16S rRNA Sequencing in the Management of Culture-Negative Pediatric Infections. *Antibiotics (Basel)*. 2022;11(2):159. Published 2022 Jan 26. doi:10.3390/antibiotics11020159.
5. El-Liethy MA, Hemdan BA, El-Taweel GE. New insights for tracking bacterial community structures in industrial wastewater from textile factories to surface water using phenotypic, 16S rRNA isolates identifications and high-throughput sequencing. *Acta Trop*. 2023;238:106806. doi:10.1016/j.actatropica.2022.106806.
6. Illumina. 16S Metagenomic Sequencing Library Preparation. Published November 27, 2013. Accessed March 29, 2024.
7. Data calculations on file, Illumina, Inc. 2022.

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

M-GL-02701 v1.0