16S Metagenomics Studies with the MiSeq® System

From DNA isolation to analysis, this simple workflow enables species-level identification of complex microbial populations in 2 days.

Introduction

Metagenomic surveys of microbial populations are often performed using the prokaryotic 16S ribosomal RNA (rRNA) gene, which contains conserved and variable regions that facilitate sequencing and phylogenetic classification. Following the complete Illumina workflow (Figure 1), 16S metagenomics studies with the MiSeq System can achieve species-level identification of microbial populations efficiently. The workflow includes DNA isolation, library preparation, sequencing, and push-button analysis, delivering an end-to-end solution for 16S metagenomics. By combining the demonstrated Illumina library preparation protocol, the MiSeq System, and simple analysis software, researchers can analyze complex microbial samples quickly and easily.

This application note provides an overview of the Illumina 16S metagenomics workflow and the results of a study that examined microbial populations in a water reservoir. This study was conducted in collaboration with the Center for Earth and Environmental Science at Indiana University–Purdue University Indianapolis (IUPUI) and Citizens Energy Group. The results reveal patterns in the reservoir's microbial community that can potentially be used to assess environmental influences on water quality.

Methods

The study used the 16S Metagenomic Sequencing Library Preparation Guide[1] to prepare sequencing libraries targeting the variable V3 and V4 regions of the 16S rRNA gene. Paired-end sequencing was performed on the MiSeq System and data were analyzed using the 16S Metagenomics App in the BaseSpace® analysis environment.

Sample Collection

Water samples were collected from the Eagle Creek reservoir in Indianapolis, Indiana at regular intervals throughout one year. Discrete samples were collected from the surface and at depths of 3 meters, 6 meters, and near the bottom floor of the reservoir.

DNA Isolation

Epicentre® DNA isolation kits deliver high-quality, inhibitor-free DNA from mixed samples of gram-positive and gram-negative bacteria derived from many environmental sources, including water, soil, fecal matter, and compost (Table 1). In this study, DNA was isolated from 27 water samples using the Meta-G-Nome™ DNA Isolation Kit[2]. Approximately 700 ng of DNA were extracted from each sample.

Figure 1: Multiplexed 16S Amplicon Sequencing Workflow

From DNA isolation to push-button analysis, the Illumina 16S metagenomics workflow provides a complete solution for complex community analyses. Sequencing with the MiSeq System delivers highly accurate data, and analysis includes classification using BaseSpace or MiSeq Reporter software.

Table 1: DNA Isolation Kits

Sample Type	Isolation Kit
Water	Metagenomic DNA Isolation Kit for Water[3]
Soil	SoilMaster™ DNA Extraction Kit[4]
Fecal matter	ExtractMaster™ Fecal DNA Extraction Kit[5]
Difficult-to-culture species present in environmental water, soil, or compost	Meta-G-Nome DNA Isolation Kit

Epicentre DNA isolation kits are optimized to isolate bacterial DNA obtained from various environments.

Table 2: MiSeq System Configurations

Flow Cell	No. of Reads	Read Length	Output	No. of 16S Samples Per Run
600-cycle V3 standard flow cell	25 M	2 × 300 bp	15 Gb	Hundreds of 16S samples
500-cycle V2 standard flow cell	15 M	2 × 250 bp	8 Gb	
300-cycle V2 micro flow cell	4 M	2 × 150 bp	1.2 Gb	Tens of 16S samples
500-cycle V2 nano flow cell	1 M	2 × 250 bp	0.5 Gb	

Library Preparation

The Illumina 16S Metagenomic Sequencing Library Preparation Guide is an easy-to-follow protocol for preparing DNA libraries. It is optimized to target the V3 and V4 regions of the 16S rRNA gene, although it can be adapted to target other variable regions. The 16S Metagenomic Sequencing Library Preparation Guide leads users through each step of library preparation, from genomic DNA to sequencing-ready libraries. All necessary reagents are listed, including the required primer sequences that target the V3 and V4 regions of the 16S rRNA gene. These primers can also be modified to target different regions of the 16S gene, or altered for custom applications. The 27 samples from the reservoir were prepared using the 16S library preparation protocol and the Nextera® XT DNA Index Kit[6] for cost-effective sample multiplexing.

Sequencing

The MiSeq System can deliver 2 × 300 bp reads and up to 50 million paired-end reads, generating up to 15 Gb of data. The flexible system enables microbiologists to scale studies from one to hundreds of samples. Micro and nano flow cell options and accompanying reagents are available to support lower-throughput experiments by optimizing sample volume and coverage needs (Table 2).

Samples from the reservoir were loaded onto a MiSeq reagent cartridge and then onto the instrument. Automated cluster generation and a 2 × 300 bp paired-end sequencing run were performed. The resulting sequence reads were equally distributed across the samples, demonstrating uniform coverage.

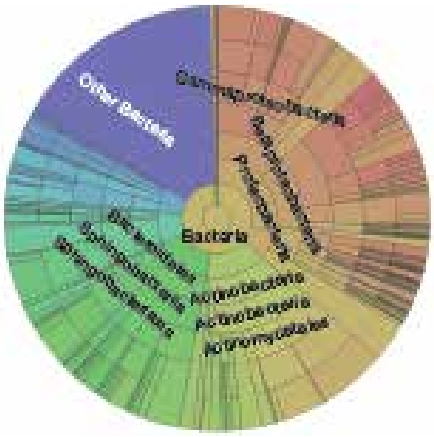
Data Analysis

Illumina has removed much of the complexity from sequencing data analysis. Following the Illumina workflow, researchers can analyze sequencing data generated on the MiSeq System either on the instrument or in BaseSpace. MiSeq Reporter software is able to analyze data on the sequencer or on a standalone computer. Alternatively, data can be transferred, analyzed, stored, and shared with collaborators in BaseSpace. BaseSpace can deliver analyzed sequences in as little as 12 hours following the 16S workflow, and BaseSpace applications (apps) provide access to a growing collection of analysis tools.

Figure 2: 16S Metagenomics App

The 16S Metagenomics App features an intuitive user interface to simplify analysis of environmental samples.

Figure 3: Relative Abundance by Taxonomic Level



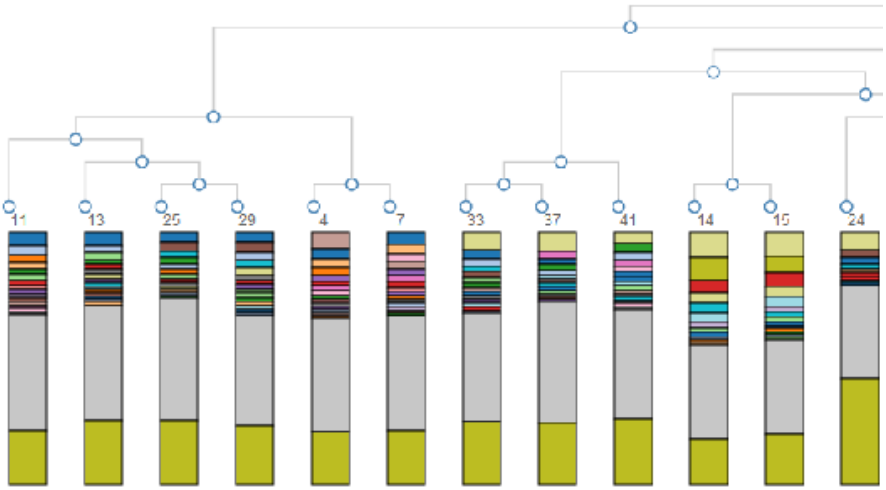
The interactive sunburst chart in the BaseSpace 16S Metagenomics App shows the relative abundance of bacterial species. Users can select any classification to magnify a taxonomic level of interest. This chart shows the relative abundance of species in a single sample from the Eagle Creek reservoir.

The reservoir samples were analyzed using the BaseSpace 16S Metagenomics App (Figure 2). The app delivers all phylogenetic data—including coverage statistics and detected species—in intuitive, easy-to-analyze reports. Sequencing reads are classified against the Greengenes[7] database, achieving up to species-level sensitivity.

Results

The 16S Metagenomics App delivers highly interactive visualizations for exploring taxonomic classifications. The sunburst classification chart provides a detailed view of the relative abundance of bacterial species within each taxonomic level. Researchers can select a category to magnify a particular level of interest and explore the diversity of any sample (Figure 3).

Figure 4: Aggregate Species Diversity



The 16S Metagenomics App delivers a dendrogram that shows a hierarchical clustering of samples based on genus-level classifications. The bar charts show the relative abundance of the classifications present in each sample. Each bar color corresponds to a unique genus.

The 16S Metagenomics App also provides an aggregate summary report so that researchers can compare the similarities and differences among samples within a project. The hierarchical dendrogram shows a clustering of samples based on genus-level classifications and the relative abundance of each (Figure 4). Detailed results and analysis from the Eagle Creek reservoir are available to view in BaseSpace[8]. Analysis of relative abundance in the reservoir revealed an increase in *Rhodococcus* species in July. Various factors may have caused the surge in *Rhodococcus* abundance, such as algaecide treatment or the use of fertilizer near the reservoir. Further analysis is required to assess the influences that contributed to changes in the community.

Conclusions

Using the 16S metagenomics workflow with the MiSeq System, microbiologists can achieve up to species-level sensitivity for metagenomic surveys of bacterial populations. In this study, the Illumina workflow was used to study microbial populations in a reservoir, uncovering shifts in community composition. This research enables biologists to investigate new methods for understanding environmental influences on water sources and water quality. 16S metagenomic studies comprise one of many applications empowered by the MiSeq desktop sequencer. Illumina solutions support researchers during every step of the process, from DNA isolation through data analysis, enabling a range of applications for microbial genomic discovery.

Learn More

For more information about the use of Illumina technology in microbial genomics, visit www.illumina.com/microbiology. To learn more about the MiSeq desktop sequencer, go to www.illumina.com/miseq.

References

1. Illumina (2013) 16S Metagenomic Sequencing Library Preparation Guide. (support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.ilmn)
2. Meta-G-Nome DNA Isolation Kit (www.epibio.com/applications/nucleic-acid-purification-extraction-kits/dna-purification-genomic/meta-g-nome-dna-isolation-kit) Accessed 07 July 2014.
3. Metagenomic DNA Isolation Kit for Water (www.epibio.com/applications/nucleic-acid-purification-extraction-kits/dna-purification-genomic/metagenomic-dna-isolation-kit-for-water) Accessed 07 July 2014.
4. SoilMaster- DNA Extraction Kit (www.epibio.com/applications/nucleic-acid-purification-extraction-kits/dna-extraction/soilmaster-dna-extraction-kit) Accessed 07 July 2014.
5. ExtractMaster- Fecal DNA Extraction Kit (www.epibio.com/applications/nucleic-acid-purification-extraction-kits/dna-extraction/extractmaster-fecal-dna-extraction-kit) Accessed 07 July 2014.
6. Nextera XT DNA Library Prep Kit | Illumina (www.illumina.com/products/nextera_xt_dna_sample_prep_kit.ilmn) Accessed 07 July 2014.
7. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72: 5069–72.
8. Project - BaseSpace (basespace.illumina.com/projects/9954952/MiSeq-v3-Nextera-XT-Metagenomics-Water-Samples) Accessed 07 July 2014.

Illumina • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

FOR RESEARCH USE ONLY

Pub. No. 1270-2013-001 Current as of 10 November 2014

illumina®