

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 Kit⁶ for cost-effective sample multiplexing.

Sequencing

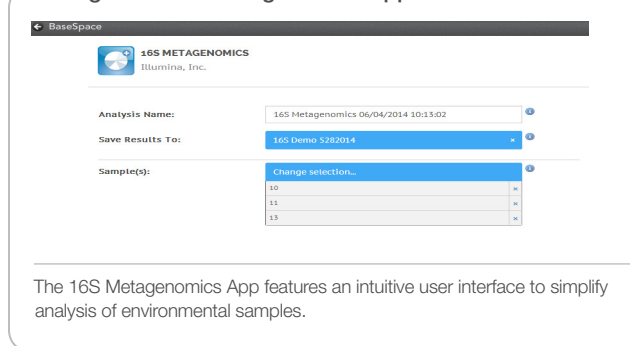
The MiSeq System can deliver 2 × 300 bp reads and up to 50 million paired-end reads, generating up to 15 Gb of data. The flexible system enables microbiologists to scale studies from one to hundreds of samples. Micro and nano flow cell options and accompanying reagents are available to support lower-throughput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

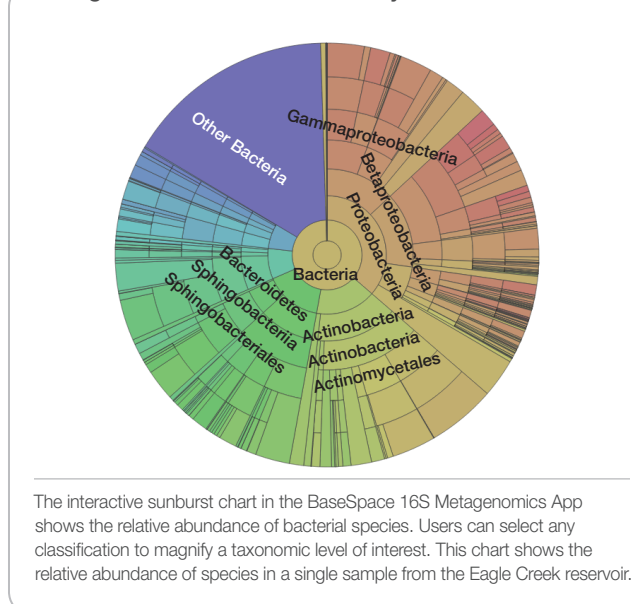
Illumina has removed much of the complexity from sequencing data analysis. Following the Illumina workflow, researchers can analyze sequencing data generated on the MiSeq System either on the instrument or in BaseSpace. MiSeq Reporter software is able to analyze data on the sequencer or on a standalone 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 Greengenes⁷ 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).

