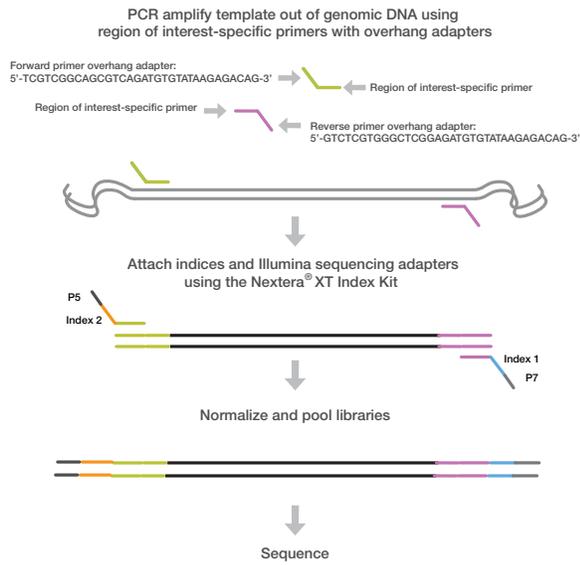




Figure 2: Simple Amplicon Workflow



User-defined forward and reverse primers that are complementary upstream and downstream of the region of interest are designed with overhang adapters, and used to amplify templates from genomic DNA. A subsequent limited-cycle amplification step is performed to add multiplexing indices and Illumina sequencing adapters. Libraries are normalized and pooled, and sequenced on the MiSeq system.

Forward primer: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3';  
Reverse primer: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'.

*mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*.

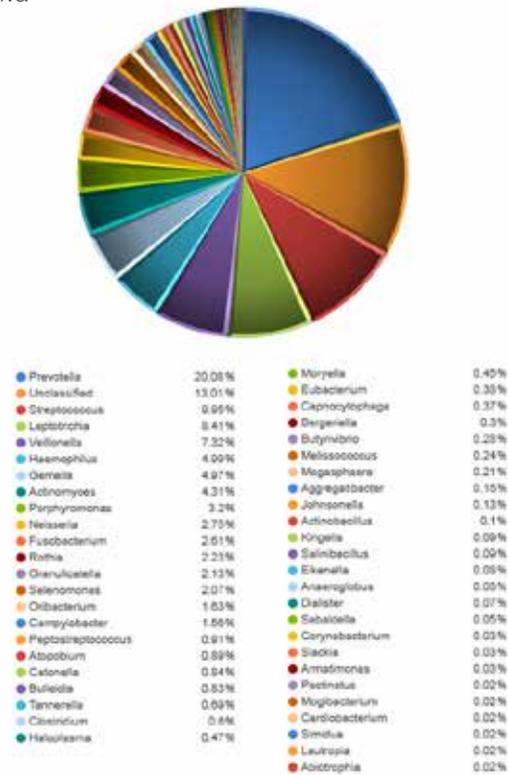
### Conclusions

Illumina's demonstrated 16S rRNA protocol can be used to sequence the V3 and V4 regions of the 16S rRNA gene on the MiSeq System, enabling genus-level identification of complex microbial populations. Simple multiplexing and increased data output allow rapid turnaround from sample preparation to results in less than 2 days. Using Illumina's MiSeq Reporter, researchers can perform complex community analysis and obtain publication-ready data. The MiSeq system can accommodate a large number of samples as well as multiple 16S regions, permitting deeper genomic scrutiny of larger metagenomic populations. Future improvements to the MSR software will feature enhanced reporting and visualization, and will include species-level sensitivity. This protocol is not limited to sequencing 16S amplicons; the workflow described here and in the demonstrated protocol document can be applied to other amplicon sequencing projects.

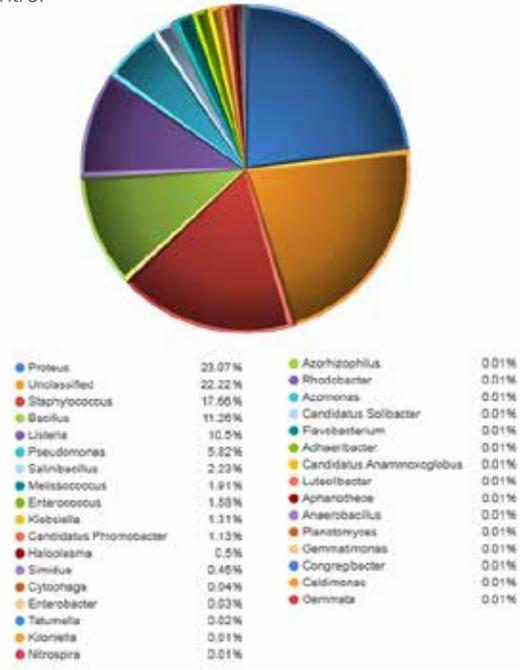
Other third-party tools are available for analyzing metagenomics samples such as Quantitative Insights into Microbial Ecology (QIIME).<sup>9-10</sup>

Figure 3: Microbial Sample Analysis

A. Saliva



B. Control



Output from the 16S rRNA Metagenomics workflow on MiSeq Reporter (MSR), showing all genera represented in the test (A) and control (B) microbial samples. DNA samples courtesy of Cleber Ouverney from San Jose State University.



