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16S rRNA Amplicon Sequencing Offers Enhanced Metagenomic Detection

The NEXTflex™ 16S V1-V3 Amplicon-Seq Kit and Illumina MiSeq's 2x300 read chemistry allow for genus-level identification

INTRODUCTION

Before the development of high-throughput methods to identify and characterize microbial populations, our understanding of the role microbes play in environmental, agricultural, and health-related settings was limited. The application of next generation sequencing (NGS) has provided an unprecedented ability to identify and categorize microbial taxonomy. Determining the complexity of species present in a sample can be achieved by sequencing a genomic region, conserved in all species, that contains evolutionarily divergent sequences that allow identification of unique taxa. A commonly used phylogenetic marker in metagenomics is the 16S ribosomal RNA (rRNA) gene. This ubiquitous locus is comprised of highly conserved regions flanking nine hyper-variable regions, referred to as V1-V9 (Figure 1). Here we demonstrate the utility of the NEXTflex™ 16S V1-V3 Amplicon-Seq Kit combined with the longer read chemistry of Illumina MiSeq (2x300) for enabling accurate identification of genera present in highly complex microbial communities across a vast number of samples.

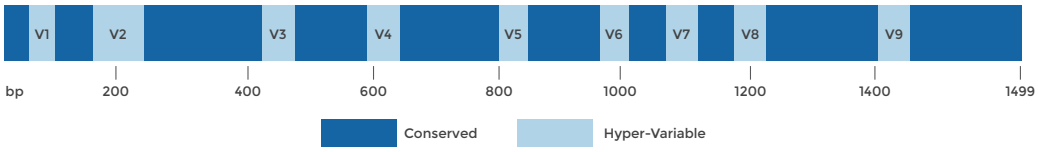

Figure 1. Schematic representing conserved and hyper-variable regions of the 16S rRNA gene.

METHODS

DNA Isolation and Microbiome Enrichment

DNA was isolated from human saliva using the QIAGEN DNeasy Blood & Tissue kit with minor modifications (1). Quality and quantity of DNA was assessed by spectrophotometry. DNA extracted from saliva was enriched for microbial DNA, and DNA quantity was determined by fluorometer.

16S V1-V3 Library Preparation

20 ng of microbial enriched DNA was used as starting material for a NEXTflex 16S V1-V3 Amplicon-Seq library prep. Targeted PCR amplification of the 16S V1-V3 region was performed using the universal primers contained in the kit, which contain library-specific overhangs and are complementary to the conserved domains flanking the hyper-variable regions of interest. After AMPure XP bead cleanup, a subsequent PCR was performed with an indexing set of primers containing Illumina flow cell binding sites, sequencing primer complementary sequences compatible with paired-end sequencing, and indexing barcodes for high-throughput multiplexing of up to 384 unique libraries (Figure 2).

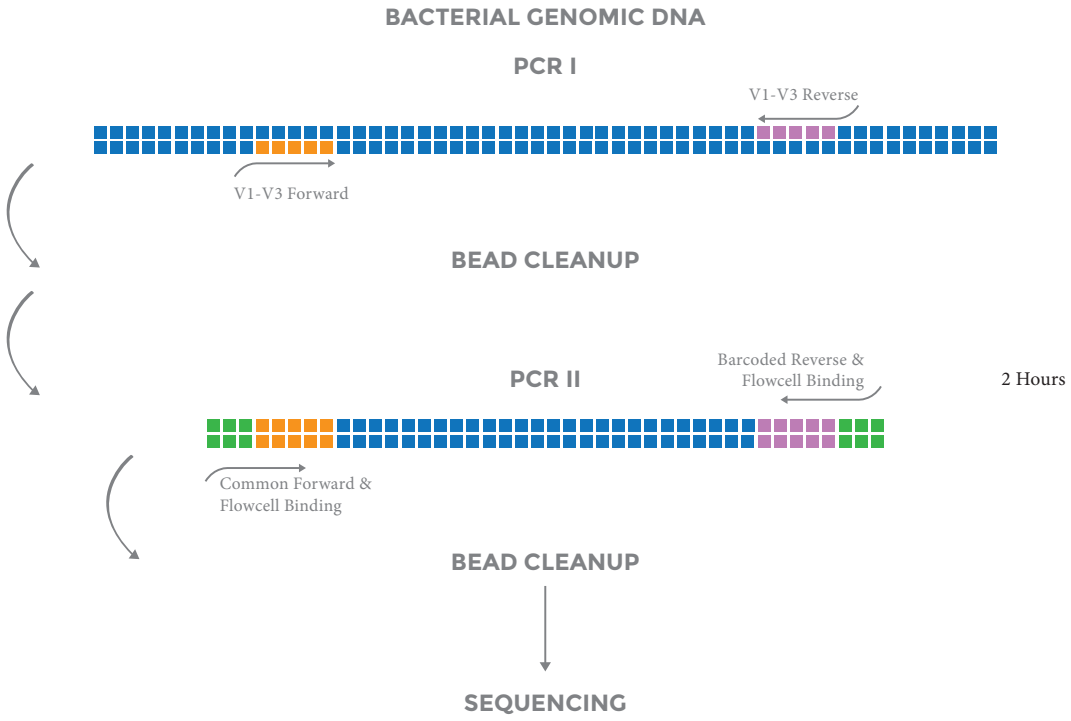

Figure 2. NEXTflex 16S V1-V3 Amplicon-Seq Kit workflow.

Sequencing and Data Analysis

Normalized libraries were clustered on-board, and paired-end sequencing was performed on the MiSeq. FASTQ files for each library were submitted to the online metagenomics analysis server, MG-RAST (2). Sequences were quality controlled and filtered before a nucleic acid similarity search against several databases of known 16S rRNA sequences was performed. Organisms detected in the total oral microbiome are shown as percent of reads mapping to genus-specific 16S rRNA references out of the total number of reads passing filter for each oral microbiome library (Figure 3).

RESULTS AND CONCLUSIONS

We explored the microbial community composition in human saliva using the NEXTflex 16S V1-V3 Amplicon-Seq Kit. High proportions of the genera *Veillonella* and *Streptococcus* were identified (Figure 3). *Veillonella* requires the presence of *Streptococcus* to adhere to the oral biofilm (plaque) and prefers lactate, the byproduct of metabolic process of *Streptococcus*, as its substrate of metabolism (3, 4). The top six genera present in this analysis: *Prevotella, Veillonella, Streptococcus, Actinomyces, Fusobacterium* and *Leptotrichia* represent abundant genera present in normal human oral microbiomes (5). Furthermore, the detection of low abundance microbes enables studies examining not only populations, but also active microbial evolution.

As a community composition study, many different 16S rRNA genes were amplified and sequenced, each with highly variable base composition, complexity and GC content (Figure 4). While the 16S rRNA region is not highly GC rich, the robust NEXTflex DNA polymerase used in the 16S V1-V3 Amplicon-Seq kit is able to amplify 45% - 65% GC content across all 16S V1-V3 regions sequenced in this experiment. Finally, the ability to detect a variety of taxa is improved by sequencing the V1-V3 regions in comparison to the V4 region alone. Using the NEXTflex 16S V1-V3 kit alone or in concert with the NEXTflex™ 16S V4 kit provides users with a time-efficient and robust method to study metagenomics, using any sample from which DNA can be obtained.

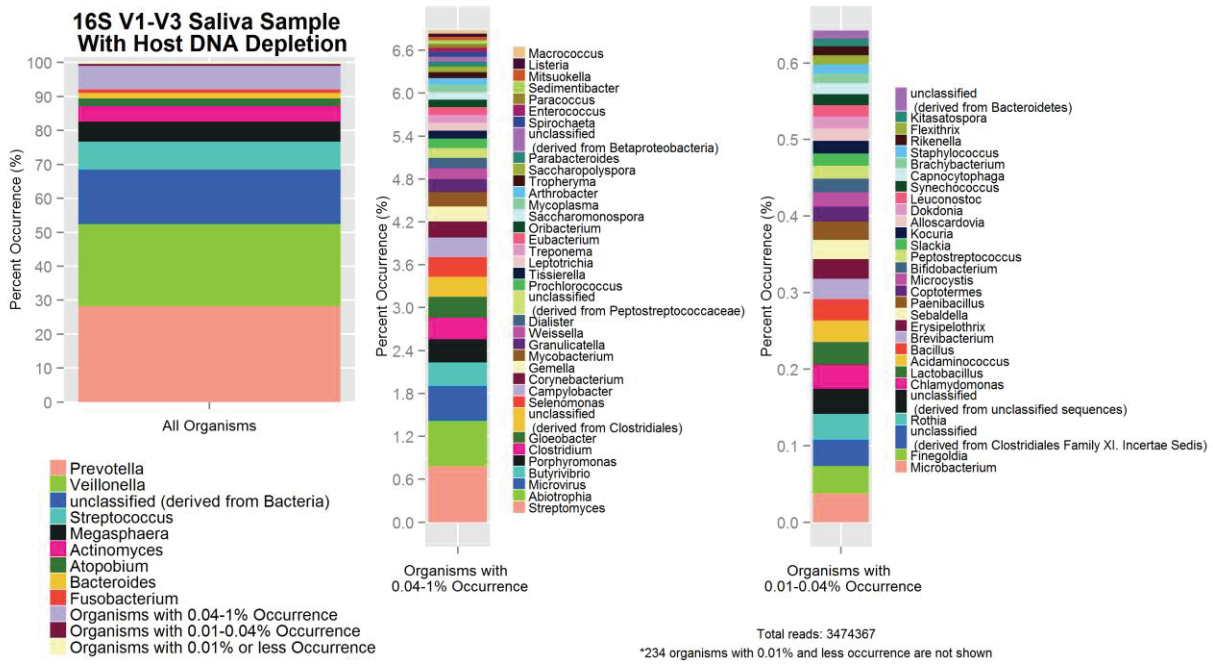

Figure 3. Genus level classification of oral microbiome from saliva sample that was enriched for microbial DNA.

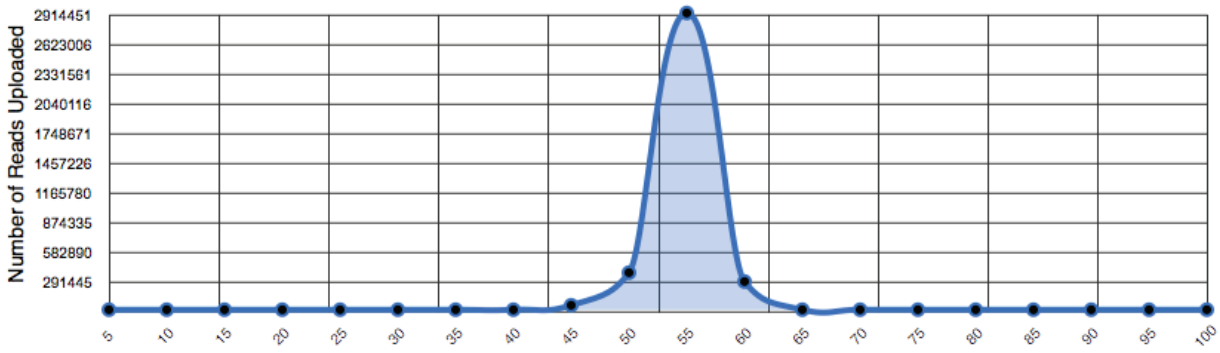

Figure 4. GC content of 16S V1-V3 PCR amplicons sequenced. Y-axis represents number of reads uploaded to MG-RAST before quality control and filtering. X-axis represents percent GC content. Plotted points represent the number of reads within a GC percentage range.

References

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