CMDC Labs

WHY DO WE PERFORM 16S RNA SEQUENCING

MAKING THE UNKNOWN KNOWN...

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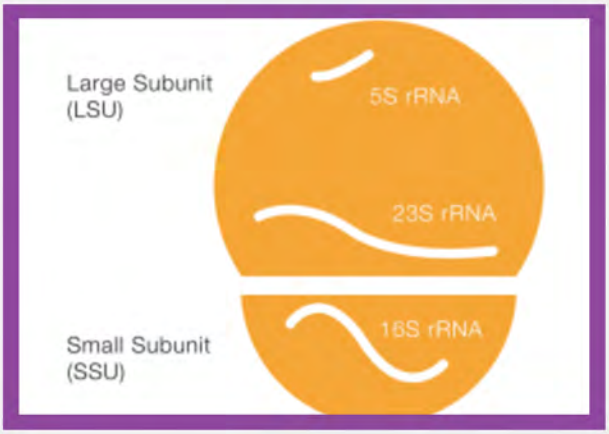
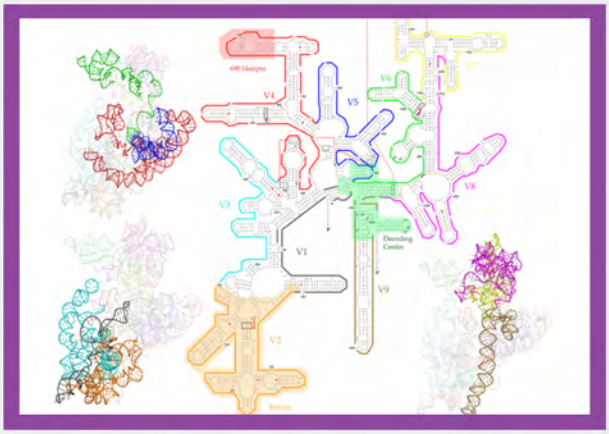
What is rRNA?

The 30S small subunit of a bacterial ribosome contains the approximately 1500 base pair 16S ribosomal RNA (also known as 16S rRNA). The role of 16S rRNA in a bacterial ribosome is depicted in Figure 1A. Nine hypervariable regions (V1-V9) with 30-100 base pair ranges are present in the bacterial 16S rRNA gene and are flanked by conserved regions (Figure 1C). The Ribosomal Database Project (RDP), Greengenes database, Silva, and Human Microbiome Project (HMP) have 16S rRNA sequences for microbial identification





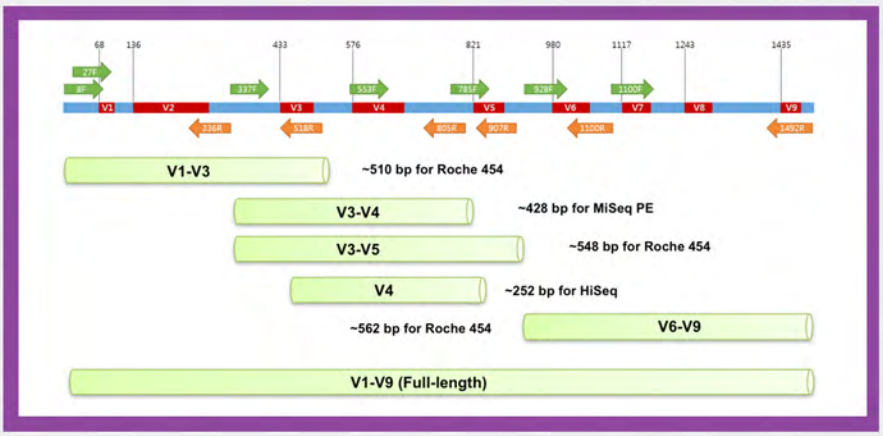


Figure 1. 16S rRNA gene. **(A)** The involvement of 16S rRNA gene in a prokaryotic ribosome. **(B)** The 2D and 3D structure of the 16S rRNA gene. Individual regions are shown in the same color (Yang et al. 2016). **(C)** The regions and primers of 16S rRNA.

16S as an rRNA Molecular Marker

The 16S rRNA gene exhibits ubiquity and evolutionary characteristics that make it possible for it to play a key role as a molecular marker in microbial ecology. 16S rRNA was first used for phylogenetic analysis by Carl Woese and George E. Fox. Furthermore, red regions (V2 and V8) have inadequate phylogenetic resolution at the phylum level, according to Yang et al. (Figure 2). Given that the green sections (V4, V5, and V6) have the shortest geodesic distances, they may be the best option for phylogenetic investigations, including those of novel bacterial phyla.

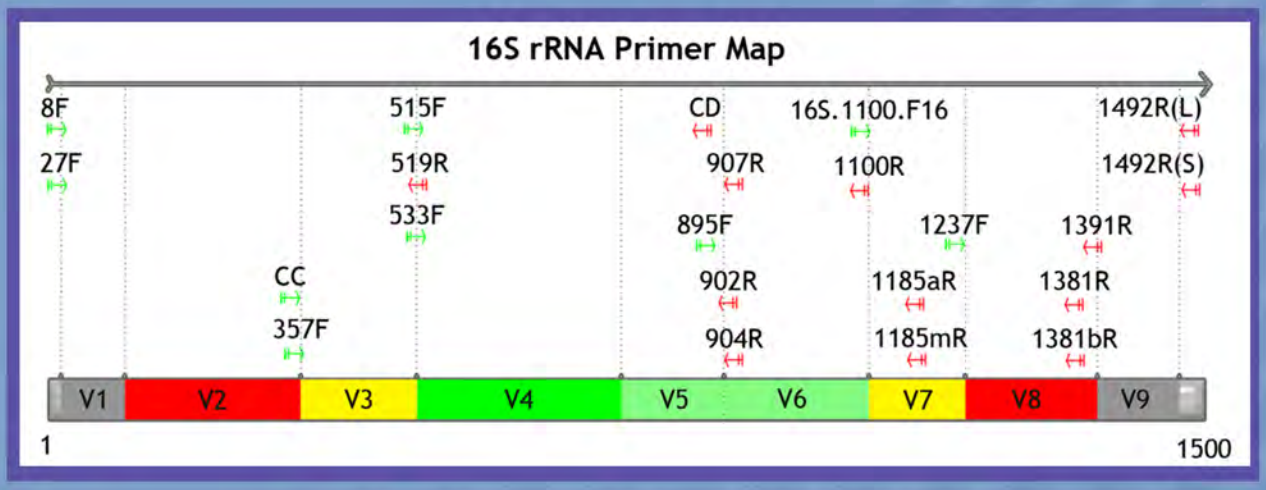


Figure 2. Illustration of different variable regions (Yang et al. 2016).

16S rRNA Sequencing

The bacterial 16S rRNA gene has various highly conserved regions inter-dispersed with hyper-variable regions. Hyper-variable sequences can provide species specific signature sequences when analyzed by 16S rRNA sequencing. This technique is widely used for identification of bacteria species in phylogentic studies. 16S rRNA sequencing is a high tech fast speed, cost-efficiency, and high-precision. It has been widely applied in basic research, as well as medical, forensic, agricultural, and industrial microbiology.

The most common method for 16S rRNA analysis use next- or third-generation sequencing technologies to read PCR products that are generated using suitable universal primers that cover one or more conserved and variable regions of the 16S sequence. For 16S rRNA sequencing, popular platforms include Pacific Bioscience, Roche 454, and Illumina MiSeq/HiSeq. The CMDC Labs platform can create full-length 16S rRNA while the first three platforms can only produce single reads that cover 100 to 600 base pairs.

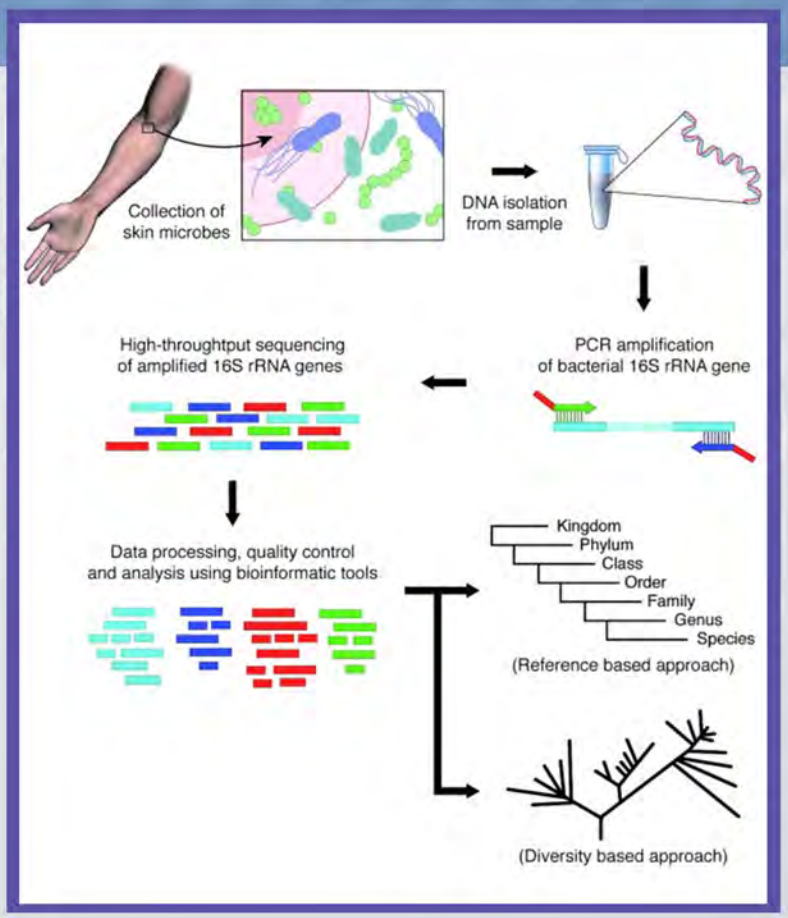


Figure 3. The workflow of 16S rRNA sequencing for human microbiome analysis.

Table 1. The common 16S rRNA sequencing regions by various platforms.

Platform	Common Sequencing regions
Illumina MiSeq	V3-V4, about 428 bp
Roche 454	V1-V3, about 510 bp; V3-V5, about 428 bp; V6-V9, about 548 bp
Illumina HiSeq	V4, about 252 bp
Pacific Bioscience	V1-V9 (full-length), about 1500 bp

ADVANTAGES AND APPLICATIONS OF 16S RNA SEQUENCING

- Identification and taxonomic classification of bacterial species
- Discovery of novel pathogens
- Phylogenetic classification
- Metagenomic survey of bacterial populations
- Clinical microbiology

REFERENCES:

1 Case R J, Boucher Y, Dahllöf I, et al. Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. Applied and environmental microbiology, 2007, 73(1): 278-288.

2 Yang B, Wang Y, Qian P Y. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. BMC bioinformatics, 2016, 17(1): 135.