Hypervariable regions matter in 16s Amplicon Sequencing

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Abstract

In recent years, 16s ribosomal RNA (rRNA) hypervariable regions have been widely sequenced across various field in an attempt to identify and differentiate among the numerous species of bacteria. From environmental microbial community studies to human and animals microbiome, high throughput sequencing of 16s rRNA gene reveals previously unknown information, allowing for a deeper understanding of the sample in study. Reviews of various scientific journals point towards a general consensus that sequencing more than one 16s rRNA region has the benefit of eliminating potential false negative results.

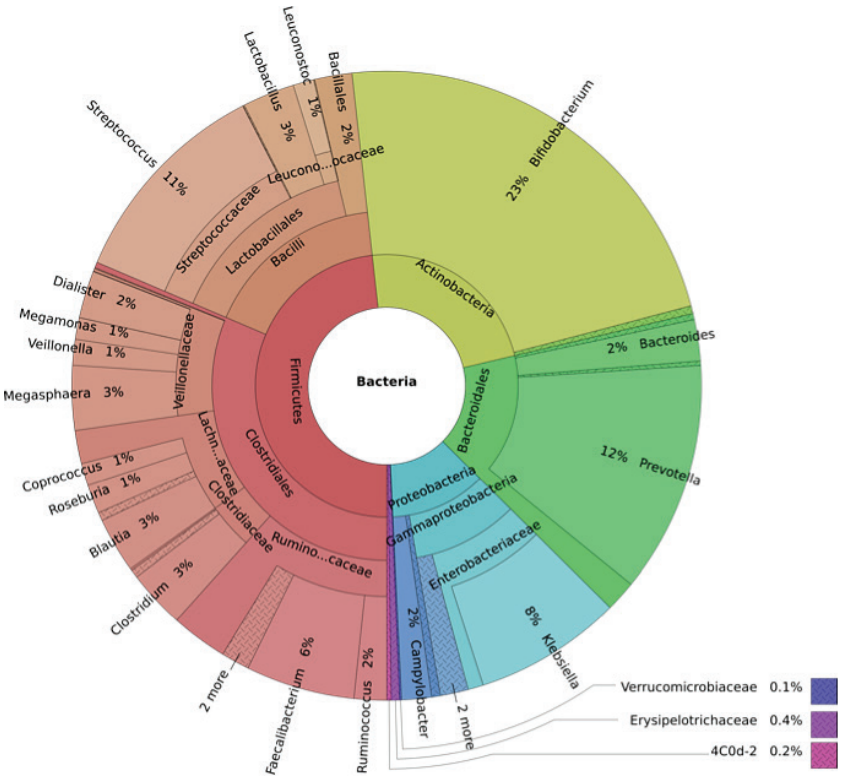


**image for illustration purposes only*

Introduction

rRNA has emerged as a useful molecular chronometers to study eubacterial evolution. The 16s rRNA gene is made up of both highly conserved and hypervariable regions. Thereby, the study of 16s rRNA genes or gene fragments, via deep sequencing of the variable regions of 16s rRNA, plays a key role to identify and characterize the microbial community in various research fields such as human microbiome project, earth microbiome project and plant microbiome studies.

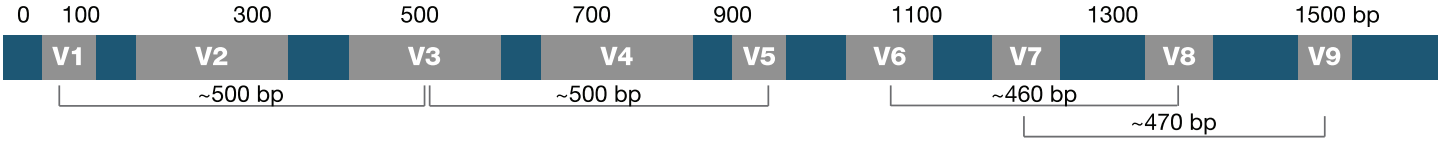
At the same time, it is also widely recognized that no primer pair is universal. A study conducted by Hong et al., (2009) reported that most of the specific primer sets, except 27F/1492R, fail to detect at least half of the bacterial compositions. One of the reasons could be due to the PCR bias, introduced by the PCR specificity, which resulted in the unevenness of frequency distribution of rRNA gene fragments among the PCR products (Sipos et al., 2007, Frey et al., 2006).

Numerous studies have documented that, depending on the sensitivity and specificity of the primer pairs, the analysis of the microbial community could vary.

Identifying Specific Bacteria Group

While the common V3-V4 target region is well-sequenced, the identification of microbial community is still not fully understood.

The following table shows a compilation of recommended 16s rRNA region for identifying specific bacteria group in different area of research.



Research Area	16s rRNA region	Targeted Bacteria Group	Citation
Pathogenic Bacteria	V1	*Staphylococcus aureus,* coagulase negative *Staphylooccus* sp.	Chakravorty, Helb et al. 2007
	V2	*Mycobacterium* species	Chakravorty, Helb et al. 2007
	V3	*Haemophilus* species	Chakravorty, Helb et al. 2007
Subgingival Microbial Communities	V1-V3	*Genera Prevotella, Fusobacterium, Streptococcus, Granulicatella, Bacteroides, Porphyromonas* and *Treponema*	Kumar, Brooker et al. 2011
	V7-V9	Genera *Veillonella, Streptococcus, Eubacterium, Enterococcus, Treponema, Catonella* and *Selenomonas*	Kumar, Brooker et al. 2011
Paddy Soil Microbiome	V1-V3	Actinobacteria (particularly in genus *Arthrobacter)*	Li, Zhang et al. 2009
	V3	β-Proteobacteria, (particularly in genus *Gallionella*) and α-Proteobacteria	Li, Zhang et al. 2009
	V3-V5	Actinobacteria (particularly in genus *Arthrobacter)*	Li, Zhang et al. 2009
	V8	β-Proteobacteria, γ-Proteobacteria and particularly in genus *Acinetobacter*	Li, Zhang et al. 2009
	V6-V8	Chlamydiae and β-Proteobacteria	Li, Zhang et al. 2009

Conclusion

Depending on the research area as shown in the papers reviewed above, the other 16s rRNA regions that were sequenced resulted in different matches of bacteria species in the database. With V3-V4 region being widely sequenced and its results well-established, it is highly recommended to sequence more hypervariable regions in combination with this region for a more complete microbial community profile.

In addition, the price of next generation sequencing has decreased over the years, thus making it more affordable for researchers to do so now, more than ever.

Hence, for an improved overall representation of the bacterial community, sequencing more individual or grouped 16s rRNA regions will allow researchers to achieve that.

Reference

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