

16S MetaVx™ Environmental Detects Greater Prokaryotic Diversity Than Traditional 16S Assay

Next generation amplicon sequencing offers the ability to rapidly analyze prokaryotic diversity in environmental samples. The 16S rRNA gene, which contains highly conserved primer binding sites and hypervariable regions (V1-V9), has become widely used for species identification and metagenomics. Optimization of 16S assays is necessary to ensure uniform amplification across species with sufficient specificity to distinguish between them^{1,2}.

Here we compare the sensitivity of GENEWIZ's 16S MetaVx Environmental service and a commonly used 16S assay. 16S MetaVx Environmental analyzes the V3, V4 and V5 hypervariable regions using two amplicons—one for the V3/V4 regions and one for the V4/V5 regions, whereas the traditional assay only amplifies the V4 region. Several environmental samples were subjected to both assays, and the amplicons were sequenced on the same run using the Illumina MiSeq with 2x250 bp configuration (Figures 1-4). Data was normalized to an average of 1 million reads per sample after sequencing. 16S MetaVx Environmental consistently detected a greater number of bacteria and archaea compared to the traditional assay, demonstrating its superior sensitivity and specificity.

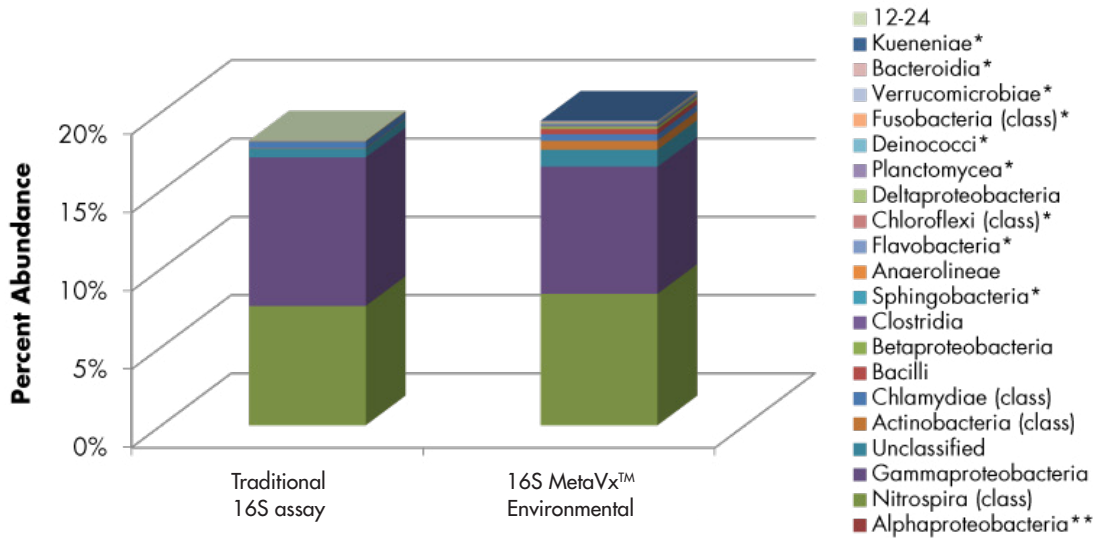


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Figure 1

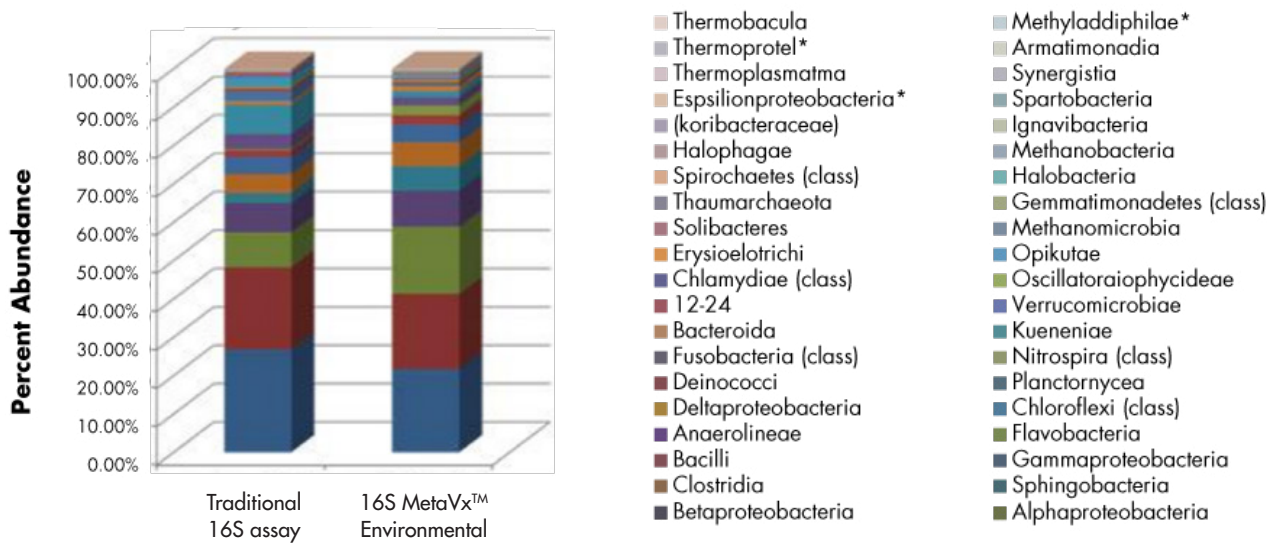


16S MetaVx detected 19 classes of bacteria, whereas the traditional assay found 11 classes.

*Detected by 16S MetaVx but not by traditional.

**The bacterial class with the highest abundance (~80% of sample in both assays) was removed for clarity.

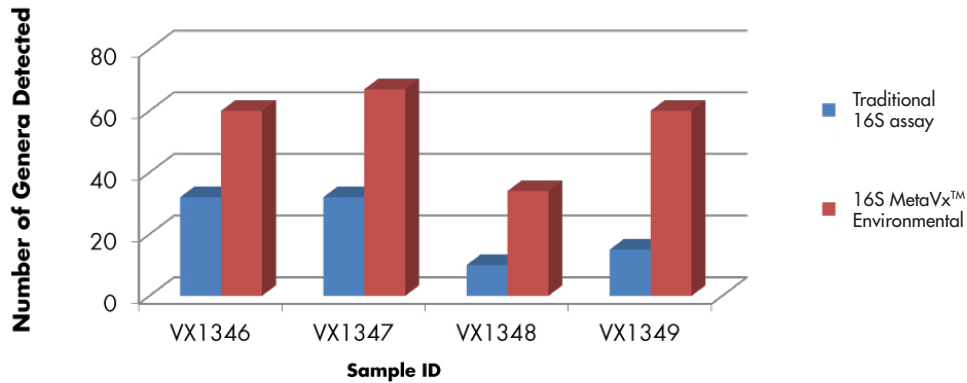
Figure 2



16S MetaVx detected 6 archaeal classes, whereas the traditional assay found only 4 classes.

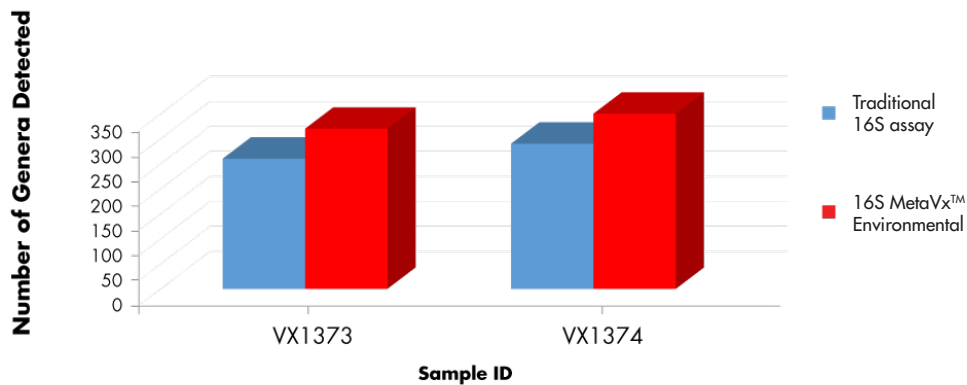
*Detected by 16S MetaVx but not by traditional.

Figure 3



16S MetaVx detected up to 4-fold more genera than the traditional assay.

Figure 4



For both samples, 16S MetaVx detected over 50 additional genera compared to the traditional assay.

References

- ¹ Baker, G., Smith, J. & Cowan, D. Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods* 55, 541-555 (2003).
- ² Wang, Y. & Qian, P. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS ONE* 4, e7401 (2009).