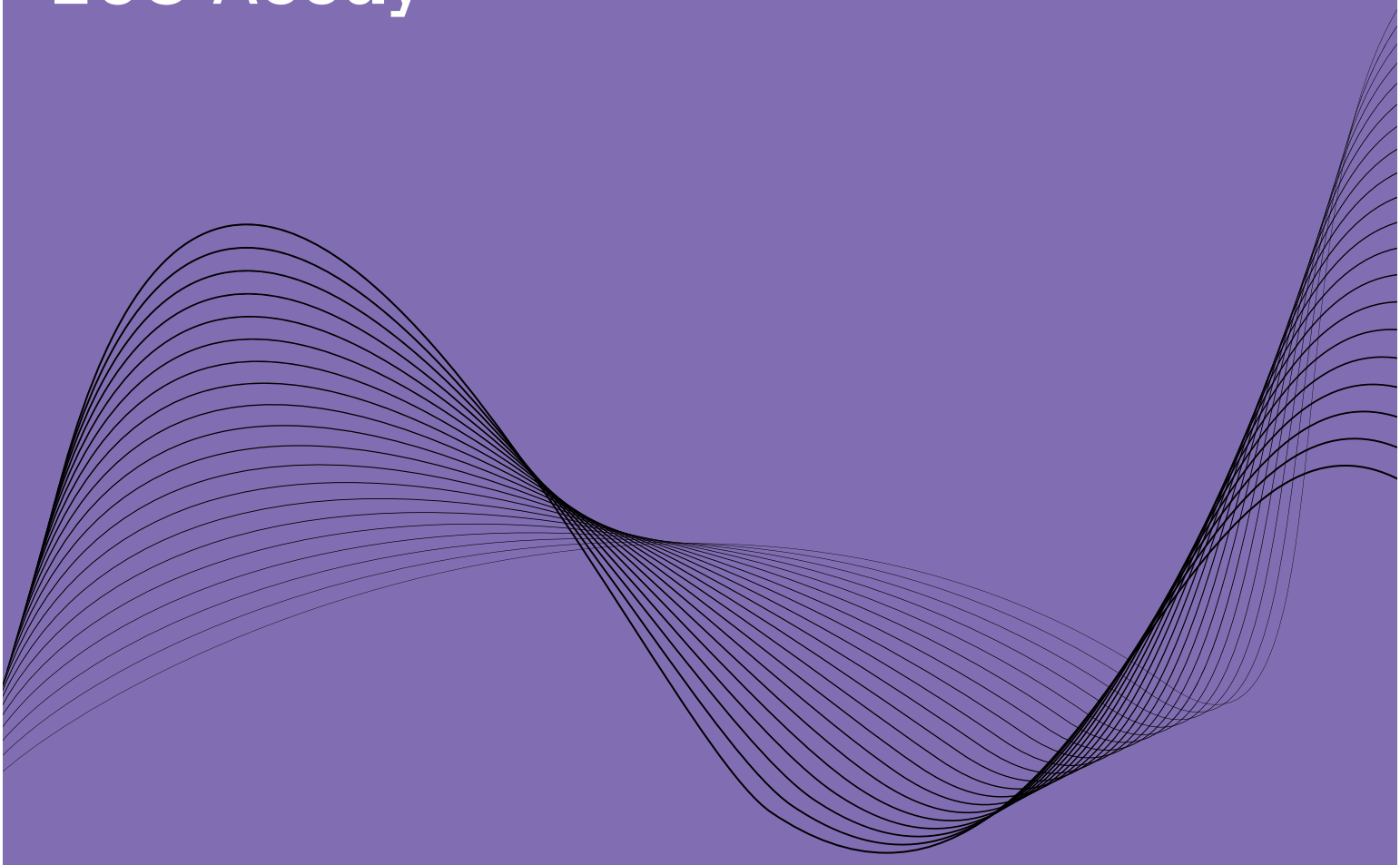
Genomics & Analytical Services

CASE STUDY

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



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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 Azenta Life Sciences' 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 2×250 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.

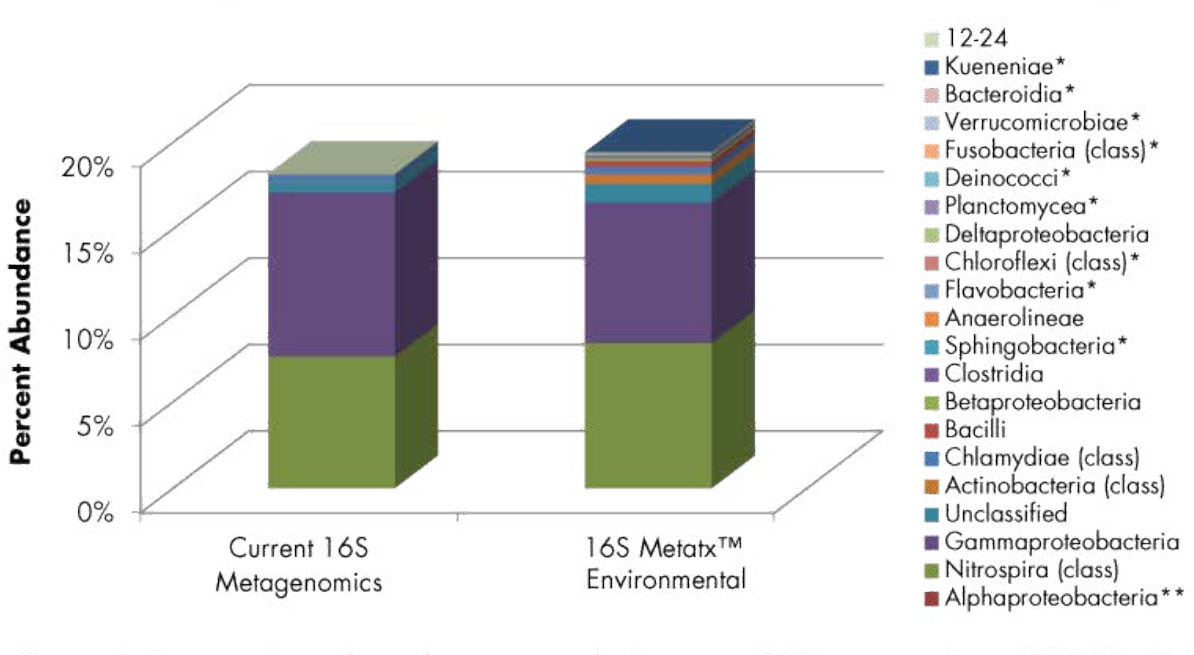

Figure 1: 16S MetaVx detected 19 classes of bacteria, whereas the traditional assay found 11 classes.
*Detected by 16S MetaVx but not by traditional.
**The bacterial class with the highest abundance (~80% of sample in both assays) was removed for clarity.

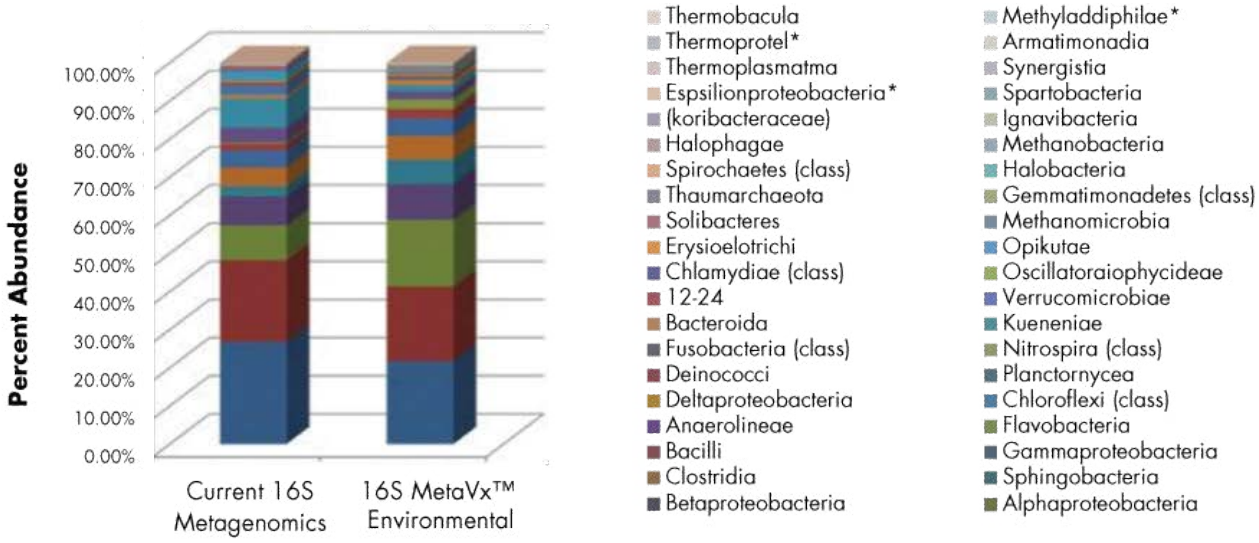

Figure 2: 16S MetaVx detected 6 archeal classes, whereas the traditional assay found only 4 classes.
*Detected 16S MetaVx but not by traditional.

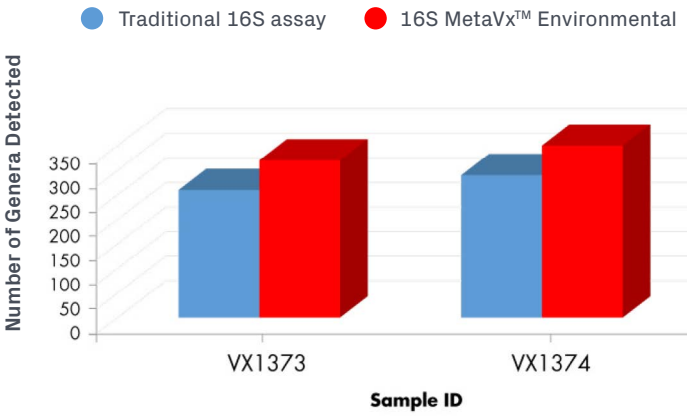

Figure 3: 16S MetaVx detected up to 4-fold more genera than the traditional assay.

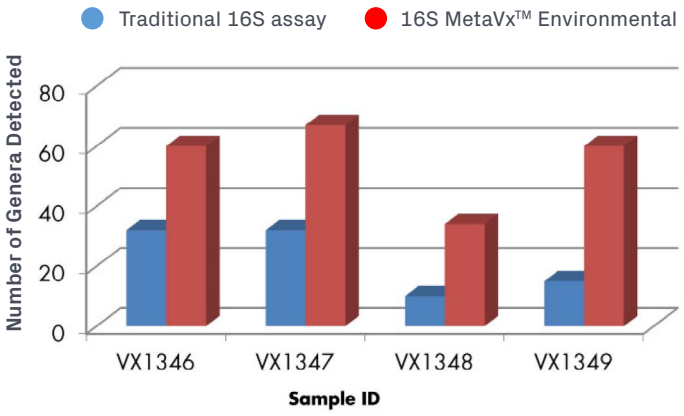

Figure 4: For both samples, 165 MetaVx detected over 50 additional genera compared to the traditional assay.

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 meta in genomic studies. PLoS ONE 4, e7401 (2009).