EnviroMail™

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Bacterial Diversity Profiling via NGS

Introduction

Bacterial diversity profiling is used for identification and classification of microorganisms present in a complex and mixed microbial community. This analysis is based on 16S rRNA gene sequencing using Next Generation Sequencing (NGS) technology and provides insight into the full microbiome.

This rapid, high-throughput and culture-free detection method has many applications in water quality management including monitoring of bacterial communities in water treatment plants, distribution systems and biofilms.

ALS offers a range of molecular tests to provide a comprehensive picture on the biological composition of the sample. Bacterial diversity profiling is part of this testing suite and is designed to estimate the relative abundance of bacteria present in the sample by sequencing seven hypervariable regions of the 16S rRNA gene.

ALS provides bacterial diversity profiling **in all water matrices (including potable, wastewaters & recycled water) and sludge/soil or sewage samples.**

Guidelines

Australian water quality guidelines for fresh, marine and recycled water provides guidance on acceptable levels of various micro-organisms.

Bacterial diversity profiling via NGS is emerging as a valuable and important screening tool in water quality assessment for regulatory compliance and to rapidly and cost-effectively identify bacterial species in polymicrobial environmental samples as a routine monitoring or surveillance tool.

Method and Reporting

ALS METHOD CODE

MP055

REPORTING

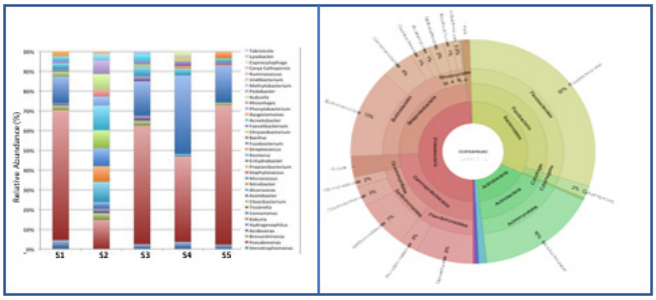
Relative proportion of each organism (%) from total DNA detected & aligned in the sequencing run.

Principle

The 16S rRNA gene, a ubiquitous gene in the bacterial genome, consists of both highly conserved regions and variable regions. The conserved regions serve as anchors for universal primers while sequencing of the variable regions allows discrimination between different microorganisms and identifies the bacteria.

Seven of nine hypervariable regions of the 16S rRNA gene are amplified by PCR and barcoded to synthesize libraries. The barcoded libraries are pooled, templated and sequenced using an automated platform.

The sequences are analyzed using the 16S data analysis pipeline within the software which aligns the sequences to the reference databases (e.g. MicroSEQ ID and Greengenes) and provides a report indicating bacteria present and their relative proportions.



Water Industry Applications

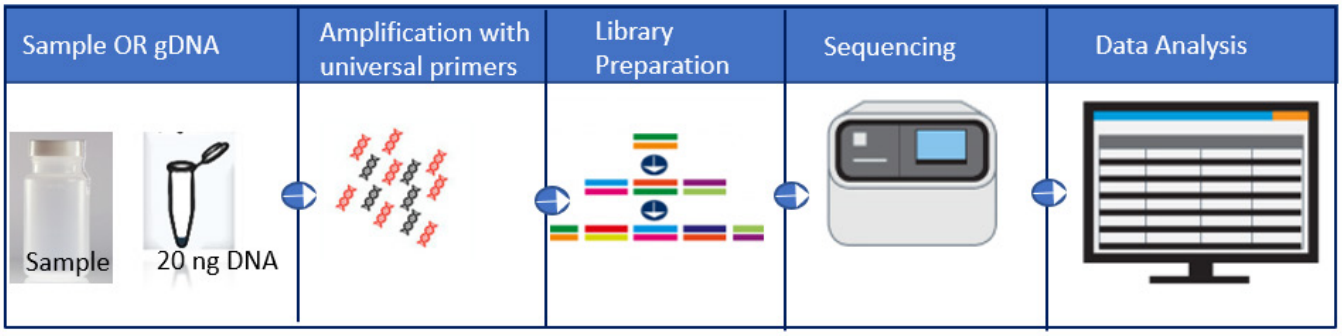
Bacterial diversity profiling can be applied to all water, sewage, soil & sludge matrices. Extracted genomic DNA can be submitted for comprehensive examination of microbial diversity. Some of the water industry related applications are:

- Assessment of bacterial community during water treatment & distribution system;
- Monitoring of bacterial re-growth and contamination in a distribution or treatment process;
- Characterization of bacterial communities in biofilms;
- Characterization of nitrifying bacteria in waste water treatment plants.
- Biocide monitoring of industrial processed waters;
- Drinking water surveillance for regular monitoring at strategic points.

Bacterial Diversity Profiling Analysis

Identification of prokaryotic species by conventional methods requires culturing of organisms in the laboratory for hours or days. Some of these organisms, such as Nitrifying bacteria, are difficult to grow in laboratory conditions. Bacterial diversity profiling eliminates the need to culture organisms in the lab and can rapidly assess microbial communities in samples containing slow-growing or uncultivable bacteria.

Nine hypervariable regions (V1-V9) present in 16S rRNA genes are considered as "fingerprint" region of DNA. This analysis amplifies multiple variable regions (V2, 3, 4, 6-7, 8 & 9) and enables the identification of broad range of bacteria to genus and species level. To perform this sequencing, water/soil or sludge samples are collected and DNA is extracted using robotic extraction systems. The extracted DNA is amplified (PCR) using primer sets targeted to the fingerprint region of interest. The amplified DNA is subjected to library preparation i.e. ligation of specific adapters oligos to the DNA fragments to be sequenced. These amplicons are loaded on to a semi-conductor chip and sequenced using pipeline performs automated analysis, annotation and taxonomic assignments. The "reads" (sequence outcome) are aligned to curated reference database to interpret population diversity within the sample.



Results include ranking of bacteria in order of relative abundance for each sample, taxonomy summary file and taxonomy summary plot. Raw sequencing files and data is available upon request. QC samples and stringent quality checks along each step of workflow ensures that output data meets the highest quality standards.

This test approach is rapid, sensitive, uses small sample size and has large sample throughput capabilities. This analysis is a valuable tool to detect bacterial indicators in water resources and can help in better management and decision making in water quality related issues.

Sampling Requirements

Holding Time:	24 hrs for samples stored at 3°C ± 2°C.
Turnaround Time:	Standard: 21 days (5 days available if required)
Sample Volume:	Water matrix: 2 L in sterile bottles Soil/Sludge/Sewage: 10g in glass bottle Extracted genomic DNA: Concentration of 1-40 ng/µl of gDNA; Total volume 20 µl per sample.
Sample Shipping & Storage	Transport sample on ice or in a refrigeration unit.

References

Vierheilig, J., Savio, D., Ley, R.E., Mach, R.L., Farnleithner, A.H. and Reischer, G.H. (2015) Potential applications of next generation DNA sequencing of 16S rRNA amplicons in microbial water quality monitoring. Water Science & Technology. 1962-1972. doi: 10.2166/wst.2015.407.