

Microbial 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 of 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. It also provides diversity estimates and functional profiling for easy comparison across temporal and spatial scales.

ALS provides bacterial diversity profiling **in all water matrices (including potable, wastewaters and 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

Method code

MM327

Reporting

Relative abundance of each organism (%), calculated using total read counts.

Diversity metrics (Shannon's Index, True Diversity)

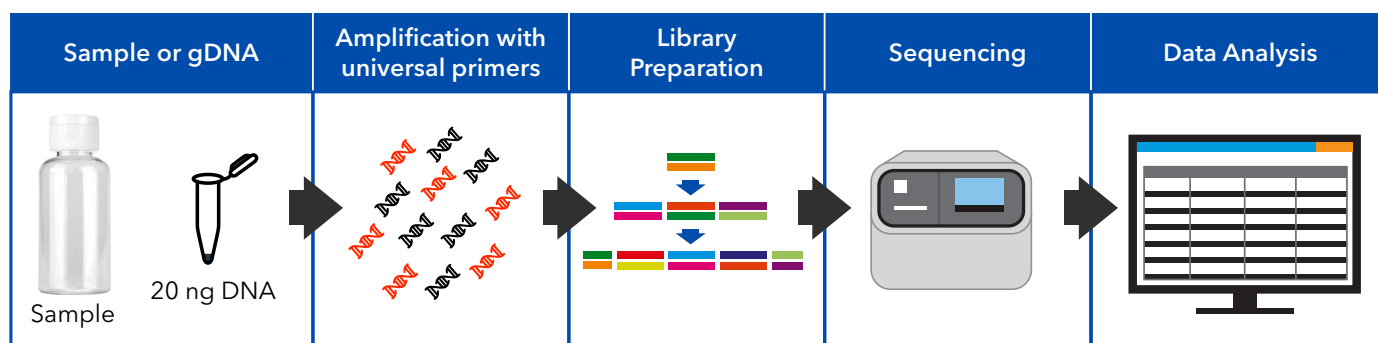
Relative proportion of functional groups based on bacterial identities.

Microbial 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. Microbial diversity profiling by NGS 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' regions of microbial genomes. This analysis uses sequence information from seven of these regions (V2, V3, V4, V6-7, V8, V9) to enable identification of a broad range of bacteria to family, genus, or species level.

The test can be performed on water, sludge, or soil samples. DNA is extracted using automated extraction systems and the target regions are PCR-amplified using specific primer sets. Amplified DNA fragments are prepared as libraries for sequencing by ligation of barcoded oligo adapters. Barcoded libraries are pooled and loaded onto a semi-conductor chip and sequenced. The sequence reads are quality trimmed and rarefied before being aligned to the reference database for taxonomic assignment. These data are further analysed using a proprietary ALS pipeline to generate a report summarising the microbial diversity profile of the sample.



Results for each sample include a list of taxonomic units found in the community, ranked by relative abundance, a pie chart summarising this, a list of functional groups linked to the taxonomic units identified, ranked by relative abundance, and a chord diagram summarising this. Raw sequencing files and data are available upon request. QC samples and stringent quality checks along each step of the workflow ensure 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. It can also be used in agriculture, food, and health settings.

Sampling requirements

Holding Time:	72 hrs for samples stored at 5°C ± 3°C.
Turnaround time:	Standard: 20 business days (5 business days available if required)
Sample Volume:	Low turbidity water matrix: 2 L in sterile bottles High turbidity water matrix: 250 mL in sterile bottles Watery Sludge: 100 mL in sterile bottle Soil/Sludge/Biofilm: 10g in glass jar Extracted genomic DNA: 20 µL of 1-40 ng/µL gDNA
Sample Shipping & Storage	Transport sample on ice or in a refrigeration unit.

References

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Liguori K, Keenum I, Davis BC, Calarco J, Milligan E, Harwood VJ, Pruden (2022) Antimicrobial Resistance Monitoring of Water Environments: A Framework for Standardised Methods and Quality Control. *Environ. Sci. Technol.* 56(13): 9149-9160.