

# BACTERIAL DIVERSITY PROFILING (bDNA)



The Australian Water Quality Centre (AWQC) is dedicated to ensuring and responding to the public health requirements relating to the provision of water and wastewater services for communities in Australia and across the world.

- Specialist water services
- Ensuring public health

## Bacterial survey employing Next Generation Sequencing (NGS)

### Overview:

The AWQC offers a full suite of molecular analyses for targeting input from problematic organisms, including vertebrates and bacteria, into water - from catchment to finished water. The AWQC is able to offer advice and solutions regarding molecular analytical approaches to determine risk and allow timely operational management decisions. Bacterial Diversity Profiling (bDNA) is part of this suite of analyses.

By using Next Generation Sequencing AWQC can provide clarity on the diversity and composition of the bacterial community present in rivers, lakes, bores, reservoirs, sewage, treated water, in short - any water matrix.

The test provides a ranking of the presence of bacterial DNA from highest to lowest prevalence (as a percentage of the total DNA extracted from the sample). Interpretational reporting in pdf/graphic formats or interactive Krona plots allow characterisation of the bacterial population, taxonomical analysis and species identification in the supplied sample. Additionally, raw NGS sequencing data and images of the bacterial diversity at any level or variable region in the taxonomy can be exported for publications and reports.

## Application and benefits

The benefits of Next Generation Sequencing (NGS) of bacteria can be realised without the need for any of the traditional microscopy or culture techniques. All that is required is a 1L water sample (or 1g of soil) from which we screen the microbial community genomes present. Our technique also has the ability to identify strains of bacteria that are usually not able to be successfully cultured, take long periods of time to grow and/or require complicated growth techniques to get a result.

The AWQC application provides the most extensive 16S rRNA coverage currently available in the Australian water industry. Our method examines seven of the nine hypervariable regions in the bacterial 16S rRNA gene - currently the most of any system and all in one efficient process. Our process compares the sequences against a defined and curated database, thus delivering the most discriminatory and sensitive bacterial NGS system available. If required, any unknown isolates can be further identified, thus creating a unique database.

Bacterial Diversity Profiling will identify the relative proportion of micro-organisms present in a mixed microbial community. This detailed snapshot of the microbial community can have many applications, for example to examine taste and odour issues in water due to biofilms, to detect the presence of problematic organisms in surface water, in groundwater bores or to assess and track the health of microbial populations in waste water treatment plants for increasing the efficiency of operational processes.

## Method

Samples are PCR amplified, with universal primer sets covering seven of the nine hypervariable regions of bacterial 16S rRNA. This enables the identification of a broad range of bacteria down to the genus/species level. The amplicons are barcoded, pooled and then sequenced. Proprietary bioinformatics software and curated reference databases are used to identify the bacteria present in samples.

Using this technology we are able to deliver the most discriminatory and sensitive bacterial NGS system available. The AWQC method employs two pieces of robotic equipment - an ION Chef<sup>(TM)</sup> and an ION S5<sup>(TM)</sup> - to create DNA chips and unique barcodes for the bacteria found in a water sample, providing detailed and reliable information.

This service provides semi-quantitative abundance estimates of the organisms prevalent in a sample by percentage and as such provides a means to compare microbial populations between samples.

## Results

Results are presented in a user-friendly report (PDF format) which includes a ranking of bacteria in order of relative abundance (% of total bacterial DNA detected). The report also includes dot-point comments against orders detected. Interpretational reporting in pdf/graphic formats allowing characterisation of the bacterial population, taxonomical analysis and species identification in the supplied sample. This is further enhanced through visualisation in the report. Interactive Krona plots are also provided to enable 'zoomable' visualisation of your results. Operational Taxonomic Unit (OTU) tables and raw sequencing files are available on request.

Note: The size of the water body, habitat of the defined species, time of year and period of collection, water flow and other hydrological factors need to be taken into consideration for optimal sampling design and subsequent bacterial diversity profiling to be effective.

### Component

- Bacterial diversity profile.

### Limit of reporting (LOR)

- Percentage (%) for each organism from the total DNA detected.

### Sampling Requirements

- Water matrix: two sterile, DNA free, sodium thiosulphate dosed 1L PET bottles.\*
- Sludge / soil / sewage matrix: minimum of 1g in sterile, DNA free, sodium thiosulphate dosed container.\*
- Extracted genomic DNA: 1-3ng of gDNA in a 10 µL volume (i.e. 3ng/µL) per sample.
- Sampler must follow DNA sampling procedure (available on request)
- No air gap for all containers.

### Holding Times

- Process within a maximum of 24hrs from time of collection.

### Turnaround Time (TAT)

- Standard: 14 days
- On request samples can be run as soon as possible on receipt.

\* If required, organisms can also be submitted as purified colonies on plates.

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