SERVICE GUIDE

Microbial Profiling Service

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SVG2402MPS

Service Guide

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1.0 Overview

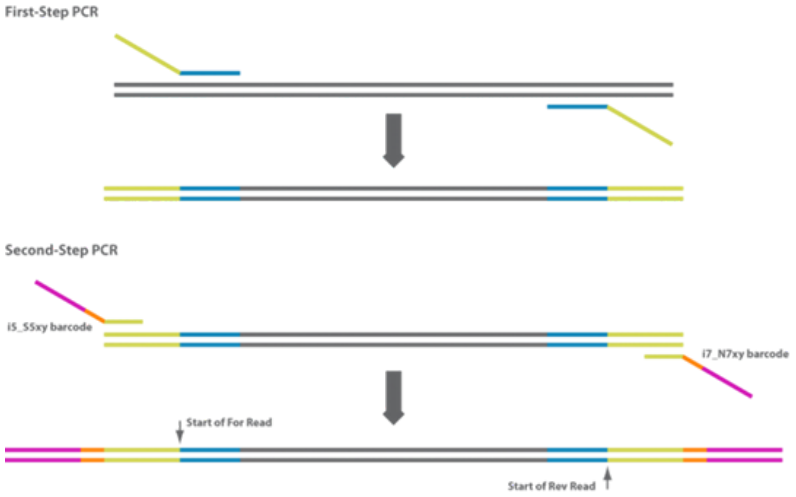
AGRF's Microbial Diversity Profiling Service is a way of identifying the relative proportion of micro-organisms present in a mixed microbial community. To do this, AGRF will take your sample, either raw sample or extracted gDNA, and PCR-amplify your selected target of interest from AGRF's available list of validated primers (Table 1).

Table 1: List of gene target regions currently available

Target Name	Forward Sequence	Reverse Sequence
16S: 27F - 519R (V1-V3)	AGAGTTTGATCMTGGCTCAG	GWATTACCGCGGCKGCTG
16S: 341F - 806R (V3 - V4)	CCTAYGGGRBGCASAG	GGACTACNNGGGTATCTAAT
ITS: ITS1F - ITS2	CTTGGTCATTTAGAGGAAGTAA	GCTGCGTTCTTCATCGATGC

AGRF will pool and sequence amplicon/s on the Illumina NextSeq™ 2000 platform, utilising Illumina's Unique Dual Indexes (UDI) and 300bp paired-end sequencing chemistry, following the workflow below (Figure 1).

Figure 1: Microbial Profiling amplicon generation & sequencing workflow



2.0 Submission Types to the Microbial Profiling Service

There are three levels of entry depending on whether the samples submitted are raw samples or extracted gDNA, and whether the client requires bioinformatics analysis. The 16S & ITS targets have no minimum sample submission.

2.1 Microbial Profiling with Extraction

This service is for clients who wish to submit a sample that requires nucleic acid extraction. This service includes:

- Nucleic Acid Extraction.
- PCR-amplification of your region of interest (including barcoding of PCR product).
- Sequencing on the Illumina NextSeq2000 platform.

Bioinformatics analysis and reporting of taxonomic distribution.

2.2 Microbial Profiling without Extraction

This service is for submitters who have already completed DNA extraction. This service includes:

- PCR-amplification of your region of interest (including barcoding of PCR product).
- Sequencing on the Illumina NextSeq 2000 platform.
- Bioinformatics analysis and reporting of taxonomic distribution.

2.3 Microbial Profiling without Bioinformatics

Microbial Profiling without bioinformatics will receive demultiplexed fastq files only.

2.4 Microbial Profiling with Bioinformatics

The bioinformatics analysis comprises multiple steps, including quality control, merging of paired-end reads, identification of Amplicon Sequence Variants (ASVs) and taxonomic classification. In addition, we offer services that include alpha and beta diversity calculations or functional inference.

2.5 Technical Considerations

In offering the Microbial Profiling Service, we will combine your indexed, amplified PCR product with other submissions, and run them together in the sequencing run. Due to inherent technical limitations and sequencing error rates, a very small number (<0.01%) of reads assigned to your sample may have originated from another sample. Likewise, very low levels of your samples may be present in another sample's data set.

If this will affect your experimental interpretation, then you should consider processing samples in isolation as a Custom project submission with AGRF (please contact us for details).

The primers we use are universal degenerate primers that have been used in several published papers. As with all universal primers, they may display a PCR bias, preferentially amplifying certain families of microbes over others. This service is not designed to identify species and should be used for family and sometimes genus identification. As such, this service should not be considered quantitative, and instead provides a way to compare microbial populations between samples. Check the literature to confirm our primers will work for you. More information and technical details can be found in our FAQs at https://www.agrf.org.au/frequently-asked-questions

Note that our 16S: 27F-519R (V1-V3) and ITS: ITS1F – ITS2R primer pair can and will amplify PLANT chloroplast or ITS region if the plant biomass is in sufficient quantities.

3.0 Sequencing Chemistry

Illumina's 300bp paired-end chemistry (600 cycles) is utilised to sequence the Microbial Profiling amplicons. The amplicons range in size from 400bp – 600bp, dependent on your specific target of interest. AGRF sequences samples for the Microbial Profiling Service by pooling similar sized amplicons together. With this in mind, submissions for > 1 target may be sequenced on separate sequencing runs.

4.0 Turnaound Time

Samples processed through the Microbial Profiling Service are pooled to enable AGRF to reduce the overall total project cost. With this in mind, samples are processed as they arrive at our facility and AGRF offers a 4 - 6 week turnaround time on this service. Please note that different targets may be sequenced on an independent sequencing run and results may vary.

5.0 Quality Guarantee

The Microbial Profiling Service guarantees each sample will obtain at least 50,000 raw reads (average ~100,000). If your sample yields less than our laboratory minimum requirement of 0.20 ng/µL of usable, indexed PCR product after we have completed our 2-Stage PCR process, and as determined by fluorometry, you will be contacted regarding how you would like to proceed. If you decide to continue with samples that did not meet our QC metric, an invoice will be generated for all samples. Alternatively, we can halt processing of the samples that did not meet our QC requirement and you may resubmit them at a later date.

If your samples have passed our PCR amplification QC and yield between 10,000 raw reads and 49,999 raw reads we will continue processing your samples and provide you the data for those samples free of charge. If samples fall below <10,000 raw reads, we will re-run your samples free of charge.

If you wish to resubmit samples that did not meet QC, please resubmit them as an independent submission. Please note that we won't hold up an existing run waiting for resubmissions. You should consider re-sampling or re-extracting DNA to ensure best sample performance. If samples fail QC again and you decide to cancel their submission, or request to resubmit a third time, an additional processing charge of $31 per sample will be applied.

6.0 Sample Returns/Discards

Samples are stored with AGRF for 1 month after you receive your data.

If you wish for your samples to be returned, you must discuss this with your account manager during quoting or contact us after you receive your data.

At the completion of your project, we can either:

- Return your samples by courier at ambient (please ask your account manager for a quote).
- Return samples by courier with dry ice (please ask your account manager for a quote).

If we are not notified within the specified time frame, samples will be automatically discarded.

7.0 Sample Submission Requirements

7.1 Samples Requiring Nucleic Acid Extraction

Online Submission:

• Submit your sample details online.
• Select: "Extraction and Microbial Profiling – gDNA" as the Service Type.
• Please complete and upload the "Template File" excel template. Note: AGRF will use the "Sample Code" to name the sequences produced from the project.
• Post/send/deliver samples to the addresses below:

Physical address (courier):
AGRF Adelaide
PLANT GENOMICS CENTRE UNIVERSITY OF ADELAIDE
HARTLEY GROVE URRBRAE SA 5064

Postal address (mail):
AGRF Adelaide
PMB1 GLEN OSMOND URRBRAE SA 5064

7.2 Extracted Samples (Purified Nucleic Acid)

Online Submission:

- Submit your sample details online.
- Select: "Diversity Profiling-gDNA" as the Service Type.
- Submission Primer Target - If your agreement is for more than one amplicon target, you will need to perform a submission for each target separately. Please note the following:
- Tube submissions - we require independent sets of tubes to be provided to AGRF per target.
- Plate submissions - we require 1 plate only.
- Submission Format – by selecting tube or plate, the "Sample File" template link will appear. Click "DownloadTemplate" and enter your sample details:
- Each sample name must be unique and can only contain alphanumeric characters and underscores.
- Save completed Template File locally, select "Browse" to upload file.
- Submit and print a paper copy of your sample submission, to be included with your sample package.
- NOTE: for multiple primer targets, please complete additional submissions.

gDNA Requirements

- Buffer – HPLC Water
- Volume – 20µL
- Concentration – 10ng/µL (or between 1 – 50ng/µL)

Submission Format

- < 24 samples – 1.5mL tube
- > 24 samples – 96 well plate (Note: if ≥24 samples are submitted in tubes, a manual handling processing fee will be added to your agreement of $1.50/tube and an additional 1.5 weeks will be added to your processing time).

Plate Format Submission Requirements:

- Leave well position A1 & A2 blank for AGRF internal controls.
- Array samples across the row (not down the column).
- Ensure the seal / strip cap is thoroughly closed on the plate prior to shipping.
- We recommend shipping plates on dry ice to ensure no cross-contamination.
- Label your plate with your Contract ID & Primer Target/s.
- Tube Format Submission Requirements:
- Please use 1.5mLsnap cap tubes. Do not use 0.2mL, 0.5mL pre-PCR tubes, strip tubes or screw cap tubes.
- Parafilm is not required.

Additional Information

- AGRF recommend that A260/A280 ratios are performed on all gDNA samples prior to submission. The ideal range is 1.6 – 1.9.
- gDNA samples should be resuspended in sterile deionised water. Buffers such as TE can inhibit PCR amplification.
- AGRF recommends you test your samples for PCR inhibition prior to submission. If you cannot get your samples to amplify, we will be unlikely to do so as well.
- We ask that you submit minimum 20uL of samples, to allow repeat amplification of your samples if required.
- If you are submitting samples for multiple targets, we request that you provide us 20µL of volume per target in separate 1.5 mL tubes or plates.
- Concentration recommendation – 10ng/uL.
- We find submissions between 1ng/uL–50ng/uL routinely work well with the service.
- Please note that very low input DNA can lead to amplification and sequencing of contaminating background products.
- Please note that high DNA concentration can also be misleading if there is a large amount of non- microbial host genomic DNA e.g. plant roots, tissue biopsies.

7.3 Packing of Samples

- Tube samples can be shipped at room temperature via express post.
- Samples in plate format, we recommend shipping on dry ice to avoid potential cross contamination of liquid between wells during transit due to air pressure changes in aeroplanes.
- AGRF can organise dry ice shipment for your samples as part of your quoted services or you can use our free shipping between nodes once a week service. For information on this service go to Free Shipping.

Shipping Address:
BRISBANE MICROBIAL PROFILING SERVICE,
AUSTRALIAN GENOME RESEARCH FACILITY
Level 5, Gehrmann Laboratories Research Rd
University of Queensland Brisbane,
QLD 4072

8.0 Results

After sequencing is complete, you will receive an email notification when your results are available to download from the AGRF website.

The results will include:

- The .FASTQ outputs of the run for your individual samples.
- An overview report that includes an Amplicon SingleVariant (ASV)* table for each sample, taxonomy summary files (at different levels, from kingdom down to family) and taxonomy summary plots. *ASV's are groups of sequences that are intended to correspond to taxonomic clades or monophyletic groups. These are provided as Excel spreadsheets (containing relative % reads) as well as charts (.html files).
- BLAST results for of each ASV sequence found in the sample. The BLAST search is optimized for highly similar sequences (megablast) and their results are provided in a file named "mg_blast.xlsx". These BLAST results serve as reference or supplementary and should be treated with caution.

- A ".biom" file containing ASV taxa that can be uploaded into MEGAN, an analysis tool for short read metagenomics data (information here).

An example of the .html bioinformatic output provided with your results is displayed in Figure 2.
Note that the "Microbial Profiling Without Bioinformatics" service will receive demultiplexed fastq files only.

Figure 2: Chart of phylum-level distribution of micro-organisms in 10 samples.