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1 Overview: Microbial Diversity Profiling at AGRF

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)	CCTAYGGGRBGCASCAG	GGACTACNNGGGTATCTAAT
ITS: ITS1F - ITS2	CTTGGTCATTTAGAGGAAGTAA	GCTGCGTTCTTCATCGATGC

AGRF will pool and sequence amplicon(s) on the Illumina MiSeq platform, utilizing Illumina's Nextera XT Index's and Paired End sequencing chemistry, following the workflow below (Figure 1).

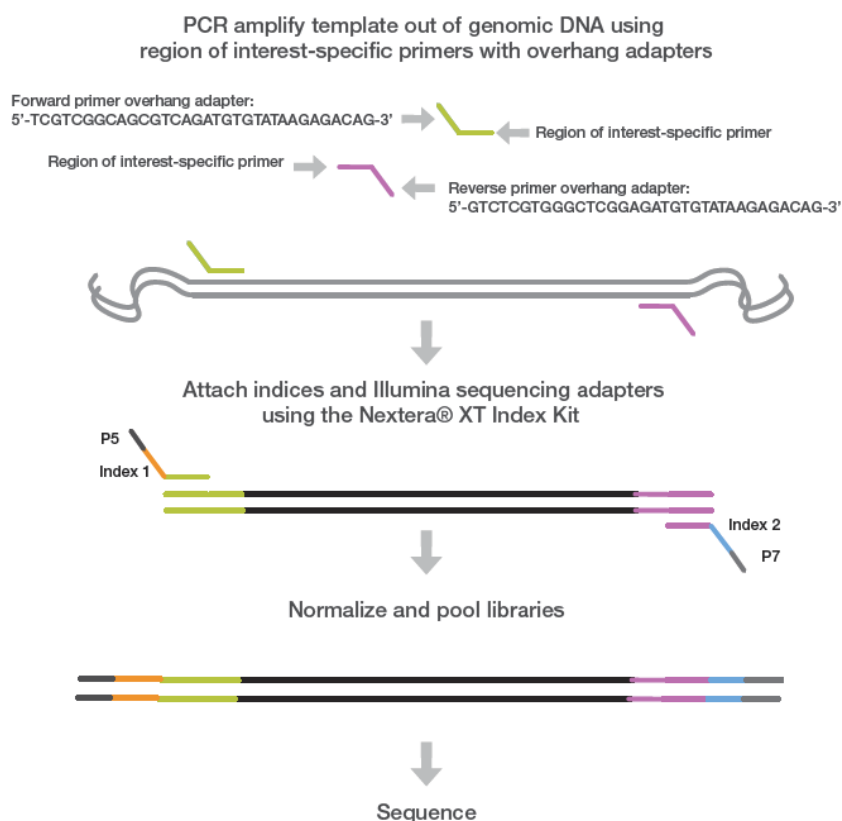


Figure 1: Diversity Profiling Amplicon Generation & Sequencing Workflow

2 Submission types to the Microbial Diversity Profiling Service

There are two levels of entry depending on whether the samples submitted are raw samples or extracted gDNA. The 16S & ITS targets have no minimum sample submission.

2.1 Diversity 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 MiSeq platform
- Bioinformatics analysis and reporting of taxonomic distribution

2.2 Diversity 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 MiSeq platform
- Bioinformatics analysis and reporting of taxonomic distribution

2.3 Technical Considerations

In offering the Microbial Diversity 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 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. 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 on our FAQ here - www.agrif.org.au/resources/next-gen-resources/divpro-faqs

3 Sequencing Chemistry

Illumina's Paired End Chemistry is utilised to sequence the Diversity Profiling amplicons. The amplicons range in size from between 400bp – 600bp dependent on your specific target of interest. AGRF sequences samples for the Microbial Diversity Profiling Service by pooling similar sized amplicon's together. With this in mind, submissions for > 1 target may be sequenced on separate sequencing runs.

4 Turn-around time

Samples processed through the Microbial Diversity 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 delivery may vary.

5 Quality Guarantee

The Microbial Diversity Profiling Service guarantees each sample will obtain at least 30,000 raw reads (average ~100,000). If your sample yields less than 0.5 ng/μL of usable, indexed PCR product 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 sample has passed our PCR amplification QC and still does not yield this many reads, we will continue processing your samples and provide you the data for those samples free of charge. If you wish to resubmit samples that did not meet QC, please resubmit them as an independent submission. You should consider resampling or re-extracting DNA to ensure best sample performance.

6 Sample Storage

Samples are stored with AGRF for 1 month after you receive your data. If you wish for your samples to be returned, we will post samples using an Australia Post Express Post satchel at ambient. Alternatively, we may return samples using Dry Ice, but this will incur a \$150 charge. If we do not hear back from you by the date specified in your data delivery email, we will discard your samples.

7 Sample Submission Requirements

7.1 Samples requiring Nucleic Acid extraction

Online Submission

- Submit your sample details [online](#),
- Select: “Extraction and Diversity 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 sample to the address 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 “Download Template” 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

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
- Label your plate with your Contract ID & Primer Target/s

Tube format submission requirements

- Please use 1.5mL snap 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 re-suspended in sterile de-ionised 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 sample to amplify, we will be unlikely to do so as well.
- We ask that you submit minimum 20uL of sample, to allow repeat amplification of your sample 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 airplanes.
- AGRF can organise dry ice shipment for your samples, please contact us for further details.

Shipping Address

MELBOURNE DIVPRO SERVICE
AUSTRALIAN GENOME RESEARCH FACILITY
LEVEL 13, VCCC
VICTORIAN COMPREHENSIVE CANCER CENTRE
305 GRATTAN STREET,
MELBOURNE, VIC, 3000

If you have any questions regarding your sample submission please e-mail brisdivpro@agrif.org.au.

8 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 Operational Taxonomic Units (OTUs)* table for each sample, taxonomy summary files (at different levels, from kingdom down to family) and taxonomy summary plots. **(OTUs) 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 OTU 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 OTU taxa that can be uploaded into MEGAN5, an analysis tool for short read metagenomics data (<http://ab.inf.uni-tuebingen.de/software/megan5/>)

An example of the html bioinformatic output provided with your results is displayed in Figure 2.

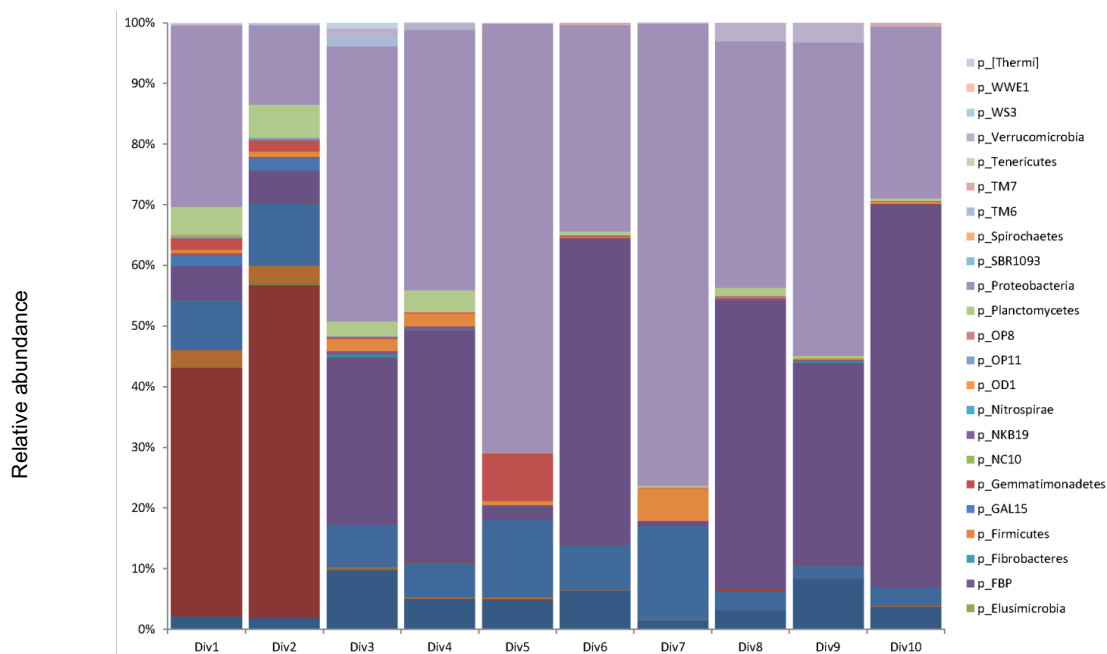


Figure 2: Chart of phylum-level distribution of micro-organisms in 10 samples