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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 microorganisms 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)	AGAGTTTGGATCMTGGCTCAG	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).

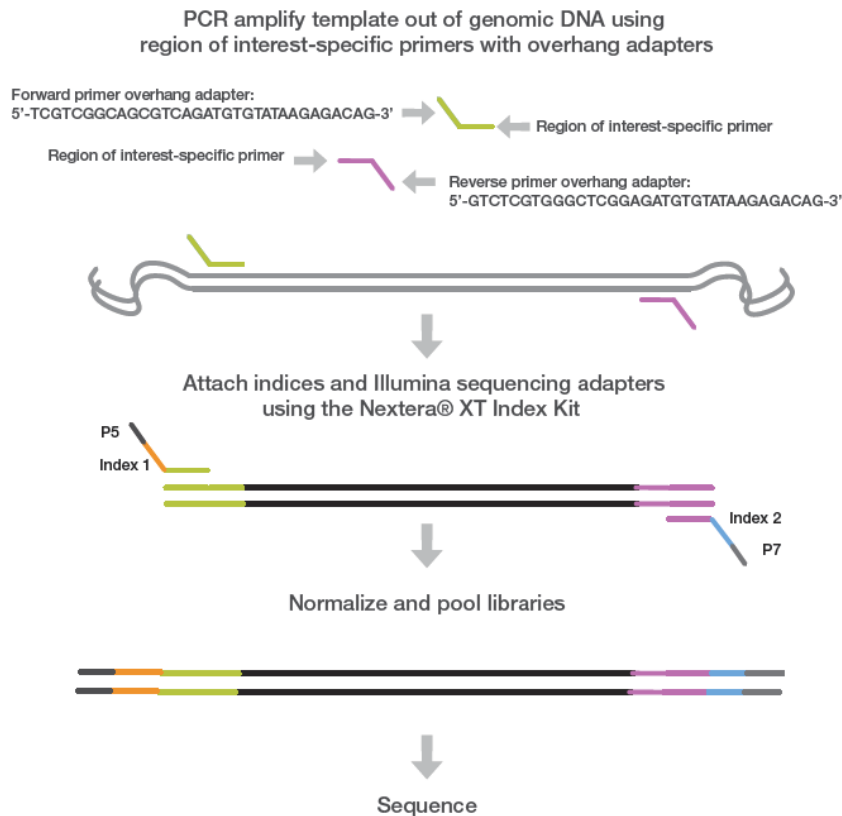


Figure 1: Diversity Profiling Amplicon Generation & Sequencing Workflow

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 raw sample. This service includes:

- gDNA 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 utilized to sequence the Diversity Profiling amplicons. The amplicons range in size from between 250bp – 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. Please contact AGRF for our run schedule if you would like to know more.

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, AGRF offers a 4 - 8 week turnaround time on this service. Please note that different targets may be sequenced on an independent sequencing run and results delivery may vary. If you would like to minimize your turn around time, please contact AGRF for our run schedule for further information.

5 Quality Guarantee

The Microbial Diversity Profiling Service guarantees each sample will obtain at least raw 30,000 reads (average ~100,000). If your sample has passed our PCR amplification QC and does not yield this many reads, please contact AGRF for a free of charge repeat. If you wish to repeat failed samples, please resubmit them as an independent submission. You should consider resampling or re-extracting DNA to ensure best sample performance.

6 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.

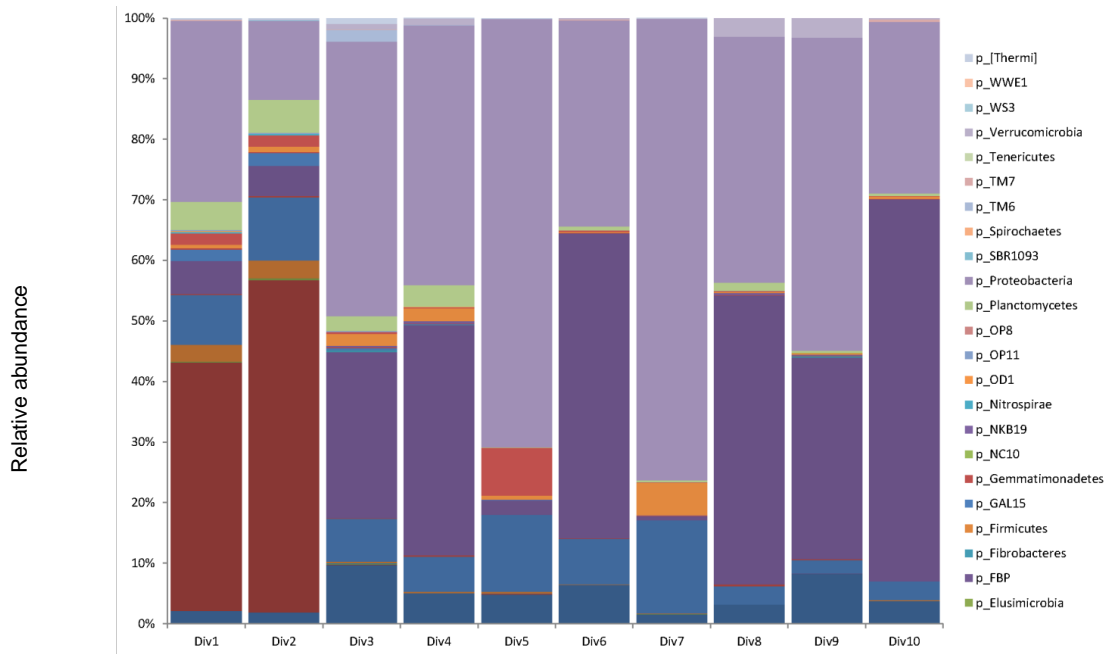


Figure 2: Chart of phylum-level distribution of micro-organisms in 10 samples

7 Physical Sample Submission Requirements

7.1 For raw samples (requiring gDNA extraction)

- Submit your sample details online, to the AGRF Nucleic acid extraction service at AGRF Adelaide.
- Please complete and upload the “Sample File” excel template. Note: AGRF will use the “Sample Code” to name the sequences produced from the project

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 For extracted samples (gDNA)

- If you are planning to sequence more than one target for any given sample, you need to submit each sample independently for each target, as they will be processed independently in our lab at different times. Please provide replicate tubes or plates for each sample and perform a unique submission per amplicon target online the AGRF website.
- For submissions of < 24 samples, please submit in 1.5mL microfuge tubes.
- For submissions of > 24 samples, please submit in either 1.5mL tubes, or a 96 well skirted 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 to avoid potential cross contamination of liquid between wells during transit.
 - Label your plate with your Contract ID & Primer Target.
- Please do not use 0.2mL, 0.5mL pre PCR tubes or strip tubes.
- 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 at least 10uL of sample per target, to allow repeat amplification of your sample if required.
- 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.
- Submit your sample details online to the AGRF Diversity Profiling-gDNA (MiSeq) service (one submission per amplicon target). Enter your sample details, and complete and upload the “sample file” Excel template.
- Include a paper copy of your sample submission sheet/s with your sample package.



Shipping Address:

BRISBANE DivPro Service
AUSTRALIAN GENOME RESEARCH FACILITY
LEVEL 5, GEHRMANN BUILDING (60)
RESEARCH ROAD
UNIVERSITY OF QUEENSLAND
ST LUCIA, QLD 4072

7.3 Sample Labelling

Please keep sample names simple. Clearly label tubes with your name and today's date.

7.4 Packing of Samples

We recommend using parafilm to seal caps on individual microfuge tubes. To prevent the sample tubes from moving during shipping, please pack the tubes with packing material such as paper towel or tissue paper.

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