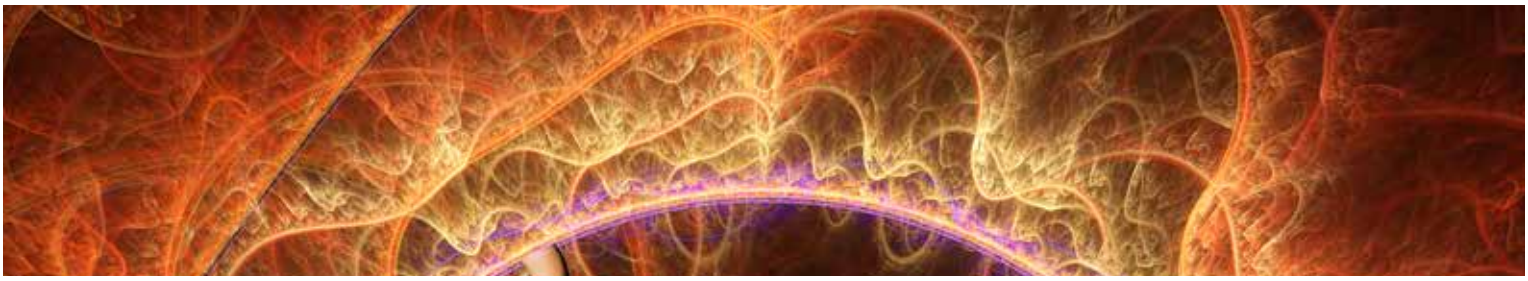
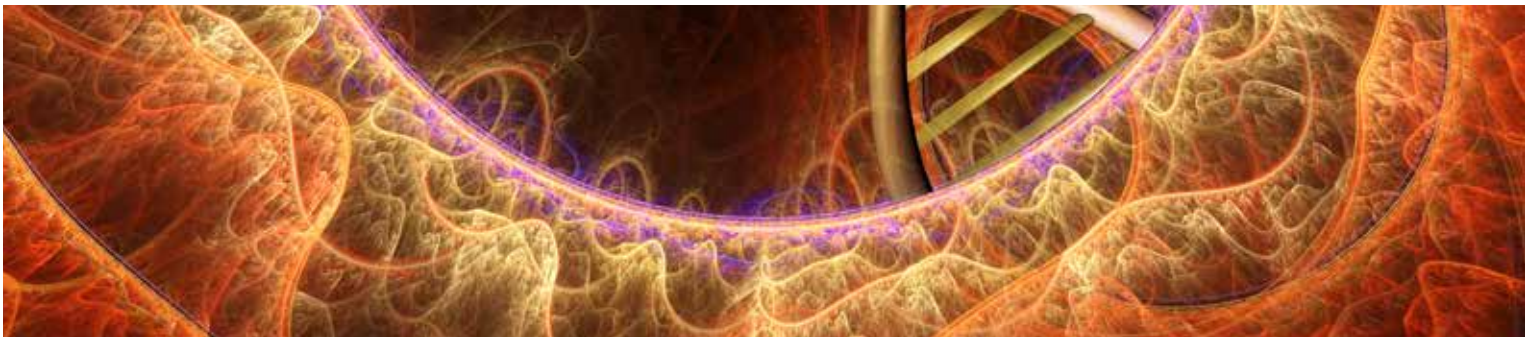


SERVICE GUIDE

Full Length 16S rRNA Gene Sequencing



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1300 247 301
www.agrf.org.au

Service Guide

Full Length 16S Sequencing

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Full Length 16S Sequencing

1.0 Overview

AGRF's full length 16S rRNA gene sequencing service is a way of identifying the relative proportion of micro-organisms present in a mixed microbial community. Short-read platforms only read partial regions of the 16S gene; however, this service uses PacBio long read technology to sequence the entire 16S gene. This provides much greater genetic information to distinguish and classify bacteria. In many cases this enables classification to the species level.

You can submit raw samples (and have them extracted through our extraction service) or genomic DNA. We PCR-amplify each sample using indexed primers (target specific sequences are shown below), pool and sequence utilising PacBio's SMRT Bell sequencing chemistry.

Figure 1: 16S gene coverage - PacBio full length vs Illumina short-read amplicons.

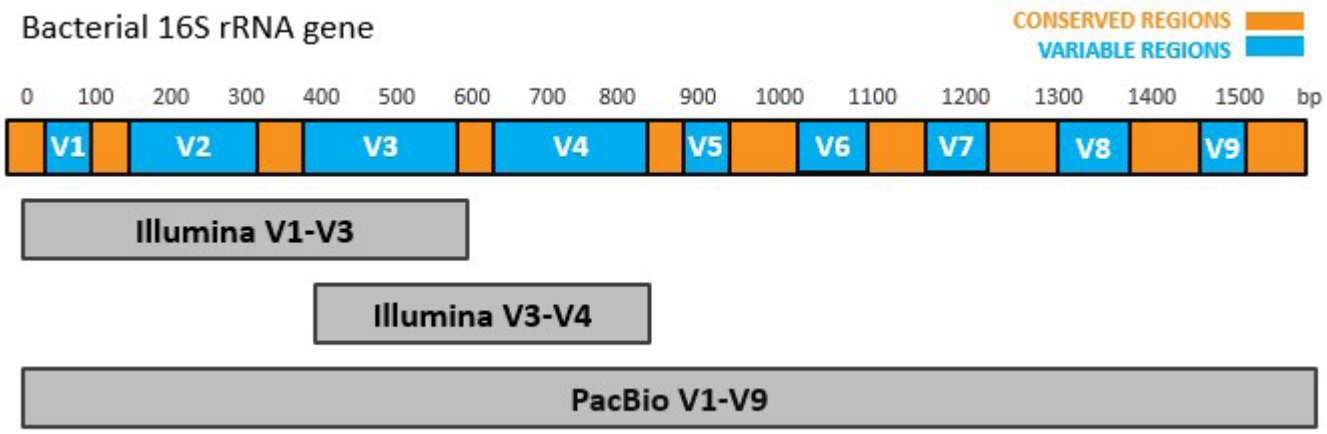

Table 1: Full length 16S primers

Primer	Name	Sequence
Forward primer	F27	5'GCATC/barcode/AGRGTTYGATYMTGGCTCAG3'
Reverse primer	R1492	5'GCATC/barcode/RGYTACCTTGTTACGACTT3'

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2.0 DNA Extraction

If your samples need DNA extraction, please enquire about our DNA extraction services.

3.0 DNA requirements

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:

- Array samples down the column (not across the row).
- Ensure the seal / strip cap is thoroughly closed on the plate prior to shipping.
- We recommend shipping plates on dry ice.

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 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 requiredConcentration 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.

4.0 Turnaround time

Samples processed through the service are pooled to enable AGRF to reduce the overall total project cost. Samples are batched as they arrive at our facility. Runs are initiated once enough samples have been collected for a run. This should result in a 6–8 week turnaround time from sample submission.

5.0 Sample submission

Before you send your samples, please ensure you have already:

1. received a formal quote from us
2. accepted the quote (provided a purchase order or return a quote acceptance form); and
3. completed an online quote request. (https://lims.agrf.org.au/ClientSubmission/Logon.aspx)

If you have not completed these steps, please do so, as this ensures that your samples can be registered by our sample tracking systems.

6.0 Shipping samples

Send your samples to the following address and include your sample submission sheet.

Physical address (courier):

AGRF Brisbane
Level 5, Gehrmann Laboratories (Building 60)
Research Road, St Lucia
UNIVERSITY OF QUEENSLAND QLD 4172

- Samples in tubes can be shipped at room temperature via express post. Samples in plate format, should be shipped on dry ice to avoid potential cross contamination of liquid between wells during transit due to air pressure changes in flight.
- AGRF can organise dry ice shipment for your samples, please contact us for further details.

7.0 Sample read output and quality checks

Expected output with good quality samples is > 8,000 reads/sample. All samples are PCR amplified and run on a gel to check for a product. If one of your samples does not produce a product, you will be contacted on how to proceed. All samples proceeding to sequencing will be invoiced. Alternatively, you may halt processing of a failed sample and resubmit at a later date.

8.0 Analysis

Analysis is included with all samples. Raw HiFi read data is also provided for clients who prefer to perform their own analyses. PacBio HiFi full-length 16S data is quality filtered and “denoised” to high quality amplicon single variants (ASV’s) using QIIME2 and DADA2. ASV classification is performed using two approaches. We perform a consensus alignment classification (using VSEARCH) against the Genome Taxonomy Database (GTDB r207). This approach should give high consistency. We also perform naïve Bayesian machine learning

based classification (DADA2) using three databases that successively fall over to the next one if a species level match is not found. In order, they are the Genome Taxonomy Database (GTDB r207), the SILVA rRNA database (v138), and the NCBI RefSeq 16S rRNA database supplemented by the Ribosomal Database Project (RDP). This should give better classification for low abundance ASV's.

9.0 Results

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

HiFi read data

- A single .BAM file containing all samples.
- Demultiplexed .BAM files (per sample).

A summary report (html):

- Project summary QC statistics.
- Table of quality filtered read counts (per sample).
- Charts and tables of the read quality/length distribution.
- DADA2 QC metrics.
- ASV count vs non-chimeric denoised reads.
- Tables of top taxonomies.

Additional Information

- Summary demultiplexing metrics.
- Rarefaction curves.
- Taxonomy classification and bar plots (using VSEARCH and Naïve Bayesian classification).
- Krona plot.
- Shared phylogenetic tree of the samples (rooted and unrooted).
- Alpha diversity (richness, Shannon index, Pielou's evenness index, Faith's phylogenetic distance).
- Beta-diversity (Jaccard index, Bray-curtis distance, weighted and unweighted UniFrac distance)
- Principle component analysis (EMPeror plots).

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Microbial Profiling Service

Figure 2: Chart of phylum-level distribution of micro-organisms in 8 samples

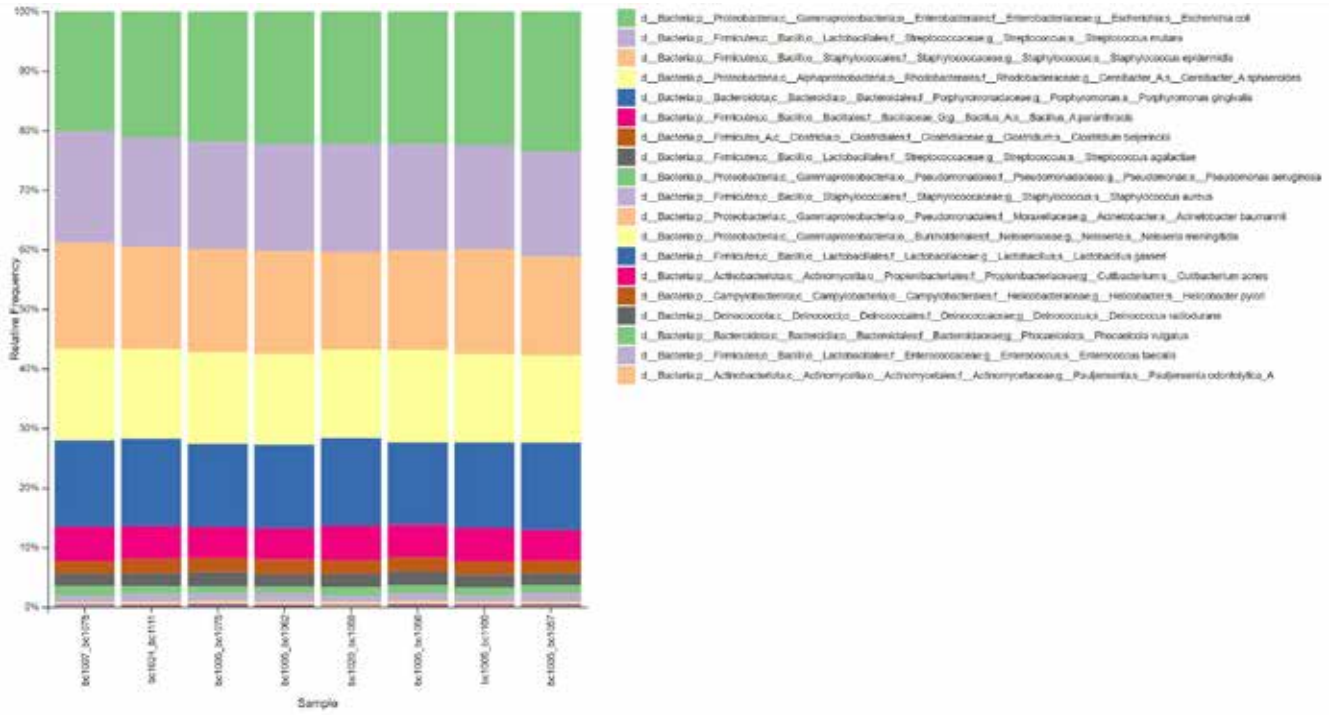

Figure 3: Krona plot of phylum-level distribution of micro-organisms in a single sample

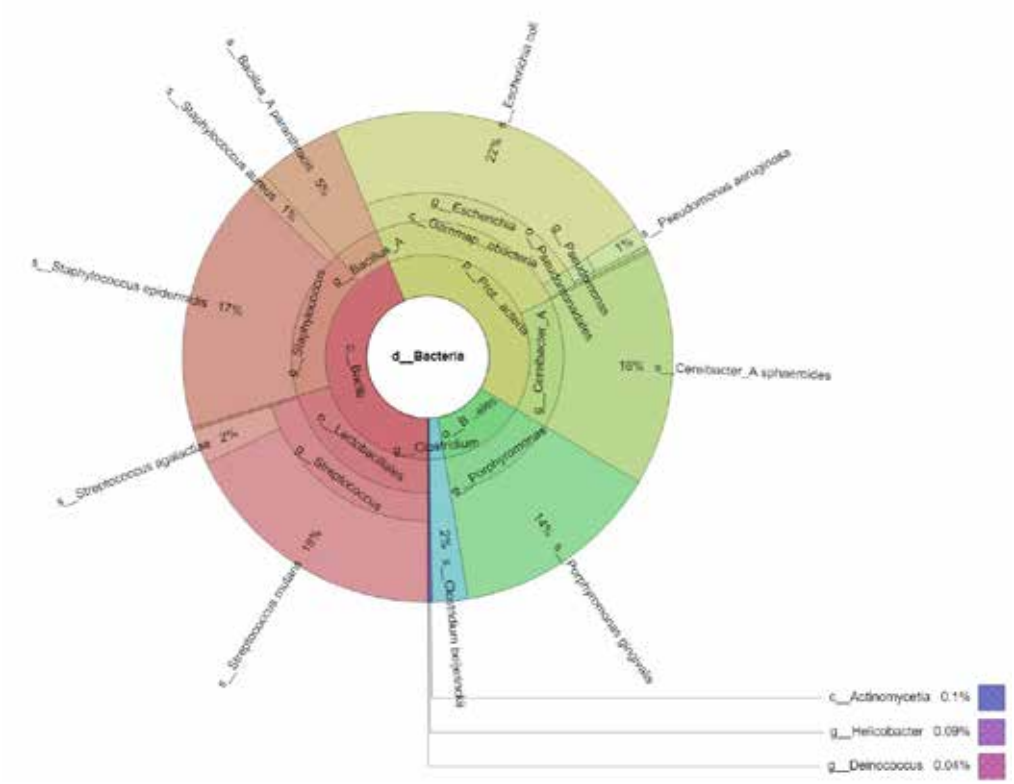

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Figure 4: Phylogenetic tree