



SERVICE GUIDE

Microbial ID via 16S Sanger

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SVG240316S

Service Guide

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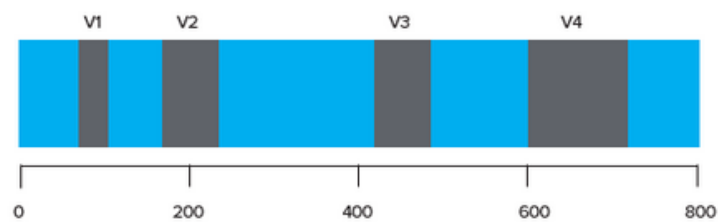


1.0 Overview

All bacteria contain the 16S ribosomal RNA gene (16S), which contains both conserved and variable regions. Sequencing of the conserved region enables identification to the kingdom level, while sequencing of the variable regions enables deeper taxonomic resolution. DNA sequencing of the 16S component of the bacterial genome has become an ideal tool for microbial identification.

AGRF's 16S sequencing service provides sequencing of the variable regions V1 through to V4 and the intervening conserved regions which allows for identification to the species level. Our service is a NATA- accredited service (ISO15189). We can screen your bacterial sample to the genus / species level via Sanger sequencing; a portion of the 16S gene is amplified and sequenced and blasted against the greengenes database.

Figure 1: Hypervariable regions within the 16S rRNA gene.



There are 9 hypervariable regions within the bacterial 16S gene, indicated in grey. The conserved regions are indicated in blue. For our 16S service we use primers that include regions V1 through V4.

2.0 Turnaround Time

Our 16S sequencing service is processed weekly - samples received by 3pm Tuesday will be processed with data available on the following Friday (3 business days).

Data is delivered via AGRF's client portal with a notification email when the data is available for download.

3.0 Sample Storage

Upon completion of sequencing, your samples will be stored at AGRF for a period of two weeks after the data is delivered. If you wish to have your samples returned post-processing, kindly inform your Account Manager during the quoting process. Please be aware that a fee will be applicable for the return of samples. The default mode of return is via Australia Post satchel at ambient temperature. If preferred, we can arrange the return of samples using dry ice. Both services will incur a fee. If no response is received by the specified date in your data delivery email, we will assume you do not require the return of samples, and they will be discarded.

4.0 Sample Submission Requirements

4.1 Sample Preparation

4.1.1. Isolates

Isolate samples should be submitted in PrepMan buffer; AGRF will supply 100 μ L aliquots of PrepMan buffer as part of our service. Please contact sanger@agrif.org.au with a suitable mailing address and number of aliquots required.

- Samples may be stored in PrepMan buffer at room temperature for up to one month.
- Select a small loopful of a single/pure bacterial colony from culture.
- Suspend the sample/colony in 100 μ L of PrepMan buffer using the 1.5 mL twist-top microcentrifuge tube provided. For health and safety reasons, refrain from suspending the sample in flip-top tubes. Additionally, avoid using thermo labels, as they may be affected during the sample heating process.
- Vortex for ~30 secs, and then heat the sample at 100°C in a water bath or heat block for 10 minutes.
- Submit the attenuated sample to AGRF Brisbane for processing (samples may be sent ambient).

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4.1.2. Genomic DNA

When submitting genomic DNA (gDNA), please provide 10 µL of DNA in a 1.5 mL Eppendorf tube. Ensure that PrepMan buffer is not included. No heating of the sample is required.

4.2 Online Submission

- Submit your sample details via our client portal.
- Select: “Sanger Sequencing” as the Service Type.
- Select: “Melbourne” as the Region.
- Select: “6387 – AGRF 16S Sequencing” as the Service.
- Select: “1.5ml Tube” as the Sample Format.
- Input the number of samples being submitted.
- Please complete and upload the “Template File” excel template.
- Please complete the Safety and Regulatory Information.
- Please complete the Payment Information.
- Please complete the Delivery and Packaging Information.

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](#).

Post/send/deliver samples to the addresses below (samples can be sent ambient):

If sending by Australia Post:

AGRF BRISBANE AGRF MICROBIAL ID VIA 16S SANGER SERVICE
GEHRMANN LABORATORIES RESEARCH RD
University of Queensland Brisbane QLD 4072

If sending by courier:

AGRF MICROBIAL ID VIA 16S SANGER SERVICE
AGRF LTD
LEVEL 5, GEHRMANN LABORATORIES RESEARCH RD
UNIVERSITY OF QUEENSLAND BRISBANE QLD 4072

5.0 Data Output

Each batch submitted will receive a batch summary report and for each sample processed, the following files are provided:

- The raw chromatogram trace file (sample.ab1).
- A trimmed FASTA formatted text file. Reads are trimmed on the basis of the quality values assigned to the basecalls (sample.fa).
- A BLAST of the trimmed FASTA file, this text file comprises the top five hits against the greengenes database (sample.bn).
- A PDF report summarising the top five hits from the BLAST file (sample.bn.pdf).

6.0 Quality Statement

Non-clinical works are performed following the strict requirements of ISO17025: 2005. AGRF Ltd is accredited in the field of Biological Testing (Scope: DNA Analysis) according to the ISO17025: 2005 standard by the National Association of Testing Authorities (NATA). Staff and analysis processes follow Standard Operating Procedures, which define responsibilities and quality checks to achieve reported standards. Compliance is monitored at regular reviews and during internal audits. All work is supervised by a person with relevant qualifications and is checked while in progress and upon completion to ensure that it meets the necessary ISO17025: 2005 standards.