

AGRF's PCR Re-Sequencing service takes your genomic DNA samples (gDNA), and oligonucleotide pairs (either designed by you, or by AGRF) to produce a PCR amplicon of targeted size, then sequence in a single- (F) or dual-direction (F/R).

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1 AGRF Sample Requirements:

- For each PCR amplification (amplicon) reaction, a 4µL sample is required at a concentration of 10ng/µL (i.e. 40ng). If you have several amplicons per sample, multiply the amount of DNA you need by the number of amplicons.
- AGRF recommend that A260/A280 ratios are performed on all gDNA samples prior to submission. The ideal optical density range is 1.6 – 1.9. Please let us know if your samples fall outside this range in the additional comments section of the online submission page.
- gDNA samples should be re-suspended in sterile de-ionised water. Please let us know during online submission if they are in a solution other than water, in the additional comments section
- Please submit samples in 96-well microtiter plates. Seal microtiter plates with a thermal film seal or adhesive foil seal prior to shipping. Adhesive plastic seals can leak during shipment and are not recommended.
- Clearly label the front and one side of the microtitre plate skirt with the following:
 - AGRF project I.D. (for example, CAGRF0123)
 - Unique plate name (if more than two sample plates are submitted)

Example – If you have 2 gDNA samples from which you would like 25 PCR amplicons produced and sequenced, AGRF would require 1000ng of gDNA in 100µL volume, for each sample.

2 AGRF Primer Requirements:

- For each PCR reaction, we require at least 2µl for both Forward and Reverse (F & R) primer at a minimum concentration of 5µM (10µM recommended)
- AGRF recommends that primers are re-suspended in sterile de-ionised water and stored at -20°C with minimal freeze-thawing
- Clearly label the side of each microtube with the following:
 - Primer name
 - Concentration
 - AGRF project I.D. (for example, CAGRF0123)

Example – If you have 2 PCR amplicons from which you would like to generate and sequence 25 gDNA samples, AGRF would require a minimum of:

- 50µL of each F primer and R primer at 5pM/µL concentrations respectively, or
 - 25µL of each F primer and R primer at 10pM/µL concentrations respectively
- NB. Micromolar concentration (µM) = picomoles/µL (pM/µL)

3 Online Submission Requirements

- Please complete the AGRF online sample submission form when sending PCR Re-Sequencing Samples to AGRF
- If you require AGRF to design primers, please complete and upload the “Gene File” excel template.
- If you have designed primers, please complete and upload the “PCR Primer File” excel template.
- Please complete and upload the “Sample File” excel template. Note that the volume and concentration cells must be numbers only (do not add the units in text in the cells)
- Note that the sample submission page will timeout after 30mins, so if you download a sample name sheet to complete the details, refresh the page before submitting.

4 Shipping Address

AGRF

Level 5 Gehrman Laboratories
University of Queensland
Research Road
St Lucia, Qld 4072