GUDSF Full Sequencing Services Guide

References:
BigDye Terminator v3.1 Cycle Sequencing Kit Protocol, Applied Biosystems 2002 (Part# 4337035A)

The success of your sequencing reaction depends upon the quality and quantity of DNA template and primer. Too much or insufficient template or primer, the presence of multiple primer binding sites or multiple primers in the BDT reaction may result in poor sequencing data. Further, contaminants such as proteins, residual salts, organics chemicals and detergents may also inhibit the reaction. Therefore, for optimal results, please submit the recommended quantity of high-quality DNA template and primer (Table 1) and also ensure that your template contains a single primer binding site.

Template quality should be examined by spectrophotometry (e.g. nanodrop) or agarose gel. If the quality is low, the template may be repurified by ethanol precipitation or with a commercial kit. PCR products should be purified prior to submission for the **Full sequencing** service.

Template quantity should be measured by spectrophotometry (e.g. nanodrop) or dsDNA intercalating dye methods. Non-specific quantitation methods such as nanodrop will also measure primers and free dNTPs in a sample.

Optional PCR purification: To ensure reaction success, the GUDSF recommends trialling a representative sample. Please contact the GUDSF prior to using this service.

Template size can be determined by electrophoresis on an agarose gel and sized against a ladder of known mass.

Table 1: Recommended input template and primer amounts:

TEMPLATE:	**Recommended quantities for a single BDT reaction.**	**Quantity recommended for Full-SEQ Service (template & primer combined in 15 µL)** [I]
PCR product 100 - 200 bp	1 - 3 ng	3 - 9 ng
PCR product 200 - 500 bp	3 - 10 ng	9 - 30 ng
PCR product 500 - 1000 bp	5 - 20 ng	15 - 60 ng
PCR product 1000 - 2000 bp	10 - 40 ng	30 - 120 ng
PCR product >2000 bp	20 - 50 ng	60 - 150 ng
Single-stranded DNA	25 - 50 ng	75 - 150 ng
Double-stranded DNA	150 - 300 ng	450 - 900 ng
PRIMER: (one primer per reaction)	3.2 pmol	10 pmol [II]

[I] Volumes can be adjusted with membrane-filtered DNase/RNase-Free Distilled Water.

[II] Approximately equivalent to 1µL of 10µM Primer

Sample submission: Please submit samples in either a 0.2mL tubes, 8-strip tubes, 48- or 96-well plate.

Sample quality: Please note, samples will not be normalised or quality assessed prior to sequencing.

Reaction: Your samples will be sequenced in the presence of a positive and negative control. Cycling conditions are as recommended by Applied Biosystems, with an annealing temperature of 50°C for 5 seconds. If you require alternate annealing conditions, please contact us prior to submission. Please note this may delay analysis.

Troubleshooting: If your samples are not producing good data, staff are happy to provide customer support.

Urgent samples: For urgent sample analysis, please contact us prior to sample submission and we will endeavour to accommodate your request.