

## DNA Sequencing Services

### Introduction:

Bangalore Genei offers DNA sequencing service for Molecular biologists. Sequence analysis is done using an automated ABI 3100 Genetic Analyser that uses fluorescent label dye terminators or fluorescent label primers. We use ABI's AmpliTaq FS dye terminator cycle sequencing chemistry which is based on Sanger's Sequencing method. Each run is termed as a 'Single Pass Analysis' where the electropherogram represents a multicolour picture of a sequence showing coloured peaks that indicate the bases.

**In automated DNA sequencing, fastidious preparation of template DNA is crucial.** Generally if the template DNA and primers are of high purity and under ideal conditions, one can expect a read of 550-650 bases, but initial 50-60 bases closer to primer annealing region may not give good readable data. The ABI Genetic analyser uses the sequencing analysis software V.5.1 with the kb basecaller which displays the quality values (QVs) for pure and mixed bases. There might be cases where the read is of short length due to the following reasons such as high background noise, co-amplification, repeat sequences of AT/GC rich template DNA. The major cause for all these may be attributed to the quality of the template DNA.

**Sequencing Service:** Bangalore Genei now offers the following services

### I) Single Pass Analysis (SPA)

Services offered for single stranded, double stranded, lambda and PCR derived templates.

#### Services Provided:

**Cat # 106024:** SPA service of PCR products with a read upto 550-650 bases using one primer and template provided by the scientist.

**Cat # 106025:** SPA service of purified plasmid DNA with a read upto 550 - 650 bases using one primer and template provided by the scientist.

#### Optional services provided :

**Cat # 106026:** Plasmid isolation service: Includes growing of a cloned *E.coli* colony with antibiotic selection, extraction and purification of the plasmid DNA. Scientist can send their clone as a plate/stab along with full information about the strain.

### II) Primer walking of constructs:

Primer walking of constructs (Sequencing of cloned inserts) is an effective strategy to sequence the entire cloned DNA insert. Bangalore Genei offers to read upto 5kb insert, bidirectionally using this technique. Initially, sequence data is obtained using a standard primer which hybridizes to the vector sequence upstream of the insert DNA. Based on this initial data a custom oligonucleotide is designed, synthesized and used to prime a second sequencing reaction. The data obtained from the second reaction overlaps with initial data and extends the sequence further downstream of the initial primer. By repeated cycles of custom oligonucleotide synthesis and DNA sequencing the cloned insert is sequenced completely in one direction. For bi-directional sequencing, a second standard primer is used which binds the vector in the other direction, the above process is then repeated and the complete sequence read for both strands.

This service includes **DNA extraction, purification, Primer designing, synthesis, desalting, sequencing and assembling** of the final sequence.

Information & sequence report will be provided to the customer at each stage of the sequencing by primer walking. In case of unforeseen problems due to GC/AT rich regions of template DNA, the customer will be informed for suggestions and billed according to the number of reaction/primer synthesized.

#### Primer service provided :

**Cat # 105292:** Custom synthesis and desalting of primers required for sequencing.

#### Purification service provided:

**Cat # 107438:** PCR product purification of samples with non specific bands using GeneiPure™ Gel Extraction Kit.

**How to process sample & primers:**

Send DNA samples along with primers. Please note that DNA and primer should be of high quality - refer note no. 1 (Sample Processing) & note no. 2 (Primer Processing). The sample should be sent along with the Application for Automated DNA Sequencing Service.

Agarose gel analysis and / or spectrophotometric check of template DNA will be done for all samples received **free of cost**. We will take up the sequencing service only if we are satisfied with the quality of DNA. Please note that if we are not satisfied with the quality / quantity of DNA samples, customer will be informed accordingly.

**Note no. 1: Sample Processing:**

Bangalore Genei recommends the use of protocol optimised by ABI for DNA preparation. Protocol is available from us on request.

Template DNA supplied should satisfy the following criteria :

1. Salt free
2. RNA free and
3. EDTA free

Template DNA must be finally resuspended in **deionised water** only.

Quantity (and concentration) of template DNA required for SPA services:

Product	Concentration (ng/ml)	Quantity (ngs)
ssDNA	50-100	600-800
dsDNA	250-500	600-800
PCR product	100-150	600-800

**Note no. 2: Primer processing:****Criteria for primers used in sequencing:**

Primer sequence selection, synthesis and purification have significant effect on the quality of sequencing data. We recommend the following criteria :

1. High Purity (either HPLC or PAGE purified).
2. Primers 18-24 bases long usually give good specificity.
3. Preferably 50 to 55% GC content.
4. No mismatch is present and no alternative hybridization sites are present in the template.
5. RAPD primers (10 mer) cannot be used for cycle sequencing reaction due to low annealing temperature.

5. For cycle sequencing, primer with melting temperature between 50 to 60° C is recommended. Avoid low melting temperature (40 to 45°C.) Melting temperature (T<sub>m</sub>) is calculated as 4 (G + C) + 2 (A + T).

6. No secondary structure particularly at the 3' end is present.

Quantity (and concentration) of primers to be provided for sequencing :

**Concentration:** At least 10 picomoles/ $\mu$ l

**Quantity:** Minimum 20-30 picomoles

If primer needs PAGE purification, scientist should send ~ 20-30 $\mu$ gs of crude primer.

**How to send samples:**

DNA must be transported in dry ice / coolant packs at sufficiently low temperature to prevent degradation. **DNA in solution should not be sent at room temperature.** DNA (especially PCR product) can be sent as lyophilised sample. Care should be taken to see that lyophilised DNA is not excessively dry as this leads to problem in reconstitution.

Customer is requested to send the Application Form duly filled in along with the samples sent for Sequence Analysis.

**Result of DNA Sequencing Service:**

Bangalore Genei will provide data of each sample in the form of (1) sequence (2) Electropherogram (3) CD.

**Delivery time: SPA services:** Approximately 1 week.

**Primer walking services:** Approximately 2 weeks for every 1 kb.

**Shipping Address:**

Kindly ship the samples to the following address

Attention:  
Bangalore Genei Services  
No. 6, 6th Main, BDA Industrial Suburb,  
Near SRS Road, Peenya, Bangalore-560 058.  
e-mail: geneiservice@sanmargroup.com.

**Note: The remaining sample will be discarded after 3 months.**