

Preparing samples for Sequencing and Electrophoresis

Sequencing and Electrophoresis

Upon submission of DNA template and primer our service can provide the DNA cycle sequencing reaction (fluorescent labelling and subsequent post sequencing clean-up) and electrophoretic separation of the labelled extension fragments.

For each sequencing and electrophoresis sample please provide the template and primer together in a total volume of 16µL.

Table 1. Recommended amounts of template and primer for sequencing reaction

Template	Quantity
Primer	3.2 pmol
Double-standed template	200-400ng
Single-standed template	50-100ng
BAC	500-1000ng
Bacterial genomic DNA	2-3 µg
PCR product:	
100-200bp	5-10ng
200-500bp	10-25ng
500-1000bp	25-50ng
1000-2000bp	50-100ng

Sample names (<10 characters long)

Please label all sample tubes with the sample name and number on the lid of the 1.5ml microfuge tube and your full name on the side of the tube.

The following characters are not recognized by the sequencing software: \ / | : * ? < > "space" so please avoid their use.

Quality of template

Template purity is a key contributor to the quality of the resultant sequence data. It is preferred that all DNA templates are post-extraction purified using a column-based DNA purification kit that ensures that residual RNA, salt, protein and other contaminating chemicals are removed. *ExoSAP-IT* for PCR purification and *TempliPHI* for plasmid amplification routinely produce very high quality DNA for sequencing as does the *Alkaline Lysis PEG precipitation method*

Quantity of template

Using the correct amount of template is critical for achieving high quality results. Too little or too much DNA will reduce the length of read and the quality of base calls. Refer to table 1 for the recommended amount of template required. The volume of template used should not exceed 6µl as we have found that less concentrated templates produce lower quality sequence reads. It is recommended

to quantitate templates by gel electrophoresis with a known mass standard or by nanodrop.

Sequencing difficult templates

The addition of 5% DMSO or 1M Betaine during sequencing cycling is recommended when sequencing templates containing secondary structure or high GC ratios. Please advise us if you require the addition of DMSO or Betaine to your sequencing reactions.

Table 2. Sequencing primers recommendations

Primer length	Primers should be at least 18 bases long to ensure good hybridisation
Single nucleotide runs	Avoid runs of an identical nucleotide, especially runs of four or more Gs
GC content	Keep the G-C content in the range 30–80% For primers with a G-C content less than 50%, it may be necessary to extend the primer sequence beyond 18 bases to keep the melting temperatures (T _m) above 50°C
Melting temperatures	For cycle sequencing, primers with T _m > 50°C produce better results than primers with lower T _m
Secondary hybridisation site	Use of primers longer than 18 bases also minimises the chance of having a secondary hybridisation site on the target DNA.
Secondary structure	Avoid primers that have secondary structure
Primer dimers	Avoid primers that can hybridise to form dimers
Concentration	Use relatively fresh stocks of primers diluted to 3.2pmol/ul in MilliQ water
Degenerate primer	Avoid degenerate primers

Table 3. Primers available from facility

Upon request, the following primers are available from the facility at 3.2 pmol/ul (um) free of charge.

Primer	Sequence
-21 M13 Primer	5' TGT AAA ACG ACG GCC AGT 3'
Reverse Primer	5' TCA CAC AGG AAA CAG CTA TGA C 3'
T3 Primer	5' ATT AAC CCT CAC TAA AGG GA 3'
T7 Primer	5' TAA TAC GAC TCA CTA TAG GG 3'
SP6 Primer	5' TAT TTA GGT GAC ACT ATA G 3'