

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 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 16uL.

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"

Quality of template

Template quality is a key contributor to the quality of the resultant sequence data. It is preferred that all DNA templates are post-extraction purified using one of the many standard, commercially available column-based, DNA purification kits, appropriate to your type of template. This ensures that residual RNA, salt, protein and other contaminating chemicals do not affect the quality of resolution (quality of resolved bases) or signal intensity of the sequence data.

Quantity of template

Using the correct amount of template is essential for high quality sequence data. It is recommended to quantitate template by gel electrophoresis with a known mass standard.

Sequencing difficult templates

The addition of 5% DMSO 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 to your sequencing reactions.

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

Primers available from facility

Upon request the following primers are available from the facility at 3.2 pmol/ul (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'