

MICROMON SANGER SEQUENCING

REQUIREMENTS FOR THE FULL CUSTOM SERVICE

DNA TEMPLATE

For each DNA template and primer combination you submit for processing, we ask that you provide the template DNA solution at the following volume and designated concentration, dependent on your template type. If you have multiple reactions for set up using the same template DNA, it can be provided in the one tube and the volume requirement can be halved.

5µl @ 100ng/µl	Single-stranded templates
5µl @ 200ng/µl	Double-stranded templates
5µl @ 10ng/µl	PCR products < 1 kb
5µl @ 20ng/µl	PCR products 1-2 kb
10µl @ 20ng/µl	PCR products > 2 kb
10µl @ 500ng/µl	Cosmids & BACs
15µl @ 500ng/µl	Genomic DNA*

*Please discuss this option with Micromon Genomics staff prior to sending any genomic DNA for Sanger Sequencing.

Please provide your template solutions in clearly-labelled 1.5 ml microfuge tubes.

These amounts are in excess of our minimum requirement but will allow us to carry out a repeat reaction if it is needed due to reaction failure or set up error. If you have less than these amounts, we will still be able to proceed but please discuss your needs with us.

It is critical that your estimate of the DNA concentration is as accurate as possible. Due to the presence of contaminants in mini-preps of plasmid DNA, even with column purification, we strongly recommend that you **estimate your template DNA concentration by gel electrophoresis** determination rather than by spectrophotometry.

PRIMER

For each template and primer combination we require **5ul of the primer at a concentration of 3-5 μM (3-5 pmol/ μl)**. In addition to high template purity, [good primer design](#) is a significant factor in successful DNA sequencing.

Please provide your primer solutions in clearly-labelled 1.5 ml microfuge tubes.

Our facility can supply the following common primers for your sequencing at no extra cost. Please check that the sequence of our in-house primers matches the priming sequence in your particular cloning vector.

M13/pUC For Primer (-21)	5' TGT AAA ACG ACG GCC AGT 3' universal
M13/pUC Rev Primer	5' TCA CAC AGG AAA CAG CTA TGA C 3'
T7 Primer	5' TAA TAC GAC TCA CTA TAG GG 3' promoter
T7 Rev Primer	5' GCT AGT TAT TGC TCA GCG G 3' terminator
SP6 Primer	5' TAT TTA GGT GAC ACT ATA G 3' promoter
T3 Primer	5' ATT AAC CCT CAC TAA AGG GA 3' promoter
CMV Forward	5' CGC AAA TGG GCG GTA GGC GTG 3'
BGH Reverse Primer	5' TAG AAG GCA CAG TCG AGG 3'