How to Prepare Samples for Macrogen Sanger Sequencing

Macrogen provides Sanger Sequencing for a full range of cloned DNA samples and PCR products. Simply place your order online and insert your samples together with a print out of your Online Order Form in a Syntezza supplied Ziploc bag. A Syntezza representative will pick up your samples and you will receive an email containing a confidential link to your sequencing results.

Templates and primers should be prepared in accordance with the following chart:

Preparing samples and primers

Template or Service	Sample Concentration	Sample Volume*	Primer Volume + Primer Concentration
Plasmid 3-4 kb	100~150ng /µl	Minimum volume 20µl	5µl + 5pmo/µl
Plasmid 4–10 kb	150ng /µl	Minimum volume 20µl	5µl + 5pmo/µl
Plasmid 10-15 kb Note: 15kb is the maximum plasmid	500ng /µl	Minimum volume 20µl	5µl + 5pmo/µl
PCR product < 300bp **Purified**	10-15ng /µl	Minimum volume 20µl	5µl + 5pmo/µl
PCR product 300-700bp **Purified**	25-50ng /µl	Minimum volume 20µl	5µl + 5pmo/µl
PCR product >700bp **Purified**	> 50ng /µl	Minimum volume 20µl	5µl + 5pmo/µl
PCR product < 300bp **Unpurified**	20-25ng /µl	Minimum volume 30µl	5µl + 5pmo/µl
PCR product 300-700bp **Unpurified**	50ng /µl	Minimum volume 30µl	5µl + 5pmo/µl
PCR product >700bp **Unpurified**	> 50ng /µl	Minimum volume 30µl	5µl + 5pmo/µl
BAC	> 500ng /µl	Minimum volume 20µl	5µl + 5pmo/µl
gDNA Full Sequencing 16S/18S/26S rRNA or ITS region (supplied in Agar Plate or Glycerol Stock)	gDNA: 30-50ng	Minimum volume 50µl	
Difficult Sequencing	100 ng /µl	Minimum volume 40µl	
Single Strand Primer Walking < 4kb	8/µg 1µg /1kb insert	Minimum volume 50µl	
Primer Walking > 4kb	Inquire for details. Requires clone in agar stab culture.		

* Macrogen uses a minimum of 5µl for the first reaction and uses the remaining sample for additional reactions, failed reaction or re-sequencing.

Templates consisting of purified plasmid DNA or PCR product, as well as primers may be submitted in individual tubes or in a 96-well plate, in solution or dried (oven dried or lyophilized). Samples submitted in solution may be sent in Nuclease-Free distilled water, 10mM Tris buffer or TE. Templates and primers may be submitted at room temperature.

If you wish to submit bacteria containing plasmid in general glycerol stock, agar-stab or agar-plate culture, please contact us for additional instruction at 02-586-7138 or by email (sequencing@syntezza.com).

Template Preparation

The success of automated sequencing critically depends on having high purity template in the correct concentration.

Plasmid DNA

There are many commercial kits available. We recommend using Qiagen miniprep or midiprep, since both methods yield consistent purity of plasmid DNA for sequencing.

Please provide DNA in the concentration range of 100ng/µl and in the amount of at least 2µg. Extra amount of DNA ensures that we have enough sample for a re-sequencing in case the first reaction fails. If samples' concentrations do not fall within this range or if you fail to provide us enough template to do the reaction, the experiment might be delayed.

PCR Fragments

The DNA must be free of contaminants, unused primers or dNTPs. PCR templates that do not undergo any kind of post PCR clean-up are not suitable for sequencing and will yield unusable sequence data. It is highly recommended that your PCR template is first observed on a gel to confirm that there is a specific product with the correct size. The Qiagen Gel extraction kit or PCR cleanup kit can be used to remove all of the unwanted elements from your template.

Host Strains

The host strain can have an impact on the quality of the template DNA prepared even using the best methods.

DH5-α host strains consistently produce good results. HB101, XL-1 Blue, JM109 and MV1190 are usually fine. JM101 is not recommended.

The growth media you use can also affect the outcome yields, while LB is usually fine.

Quantitation

Sequencers are able to handle a wide range of DNA concentrations. However, with very low amounts of DNA the data quality will be significantly affected. Using UV absorbance to quantitate dilute DNA solutions tends to give widely inaccurate results.

A preferred method to quantitate DNA is to run an aliquot on a mini-gel and compare the intensity to the control of a known concentration. There are also concentration ladders that are commercially available. For each reaction, please provide at least 20ng/µl solution in deionized water. Please provide at least 10µl additional sample for any possible re-sequencing.

Macrogen strongly recommends Gel Electrophoresis rather than Nano-drop for quantification.

Primers Preparation

Macrogen provides universal primers at no extra charge (see table below). If you prefer your own custom primer, include your primer in a tube with your order at a concentration of 5-10 pmole/ µl=60 ng/µl, in deionized water at volume of greater than 20µl. A customer supplied primer should be purified, or at least desalted. Crude primers generally do not work well for sequencing.

Primer Considerations:

- High purity
- No secondary priming sites or mismatches
- Length 18-25 bases
- GC% content between 40% and 60%
- Tm (melting temperature) between 55°C and 60°C
- No significant hairpins (>3bp)
- Free of salts, EDTA, or other contaminants

Submitting Samples in an Individual Tubes

We recommend using a 1.5µl micro-centrifuge tube. Free re-sequencing is provided for samples submitted in Individual tubes.

Submitting Samples in a 96-well Plate

We recommend placing your Template samples in a 96-well plate secured with 8-strip caps.

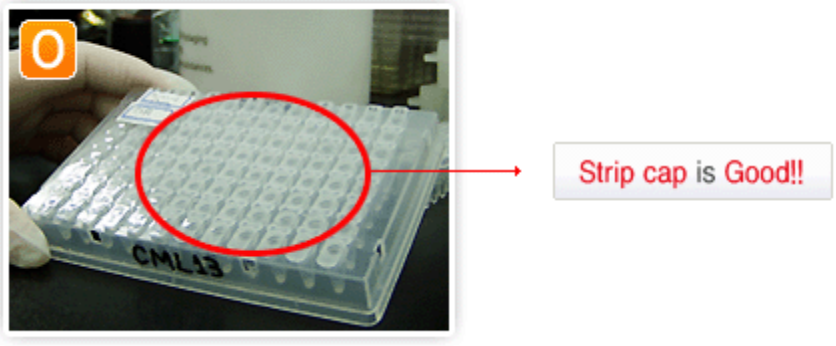
Identify on your order the universal primer from Macrogen's library you wish to use or submit with your plate of samples a tube containing your preferred custom primer.

The fee for sequencing an entire plate with the same primer is 1,045 NIS. The fee for using multiple primers for the same plate is 1,558 NIS.

Samples supplied in a 96-well plate cannot be re-sequenced.

Please maintain as consistent a sample concentration as possible across the plate wells to control well-to-well variation so as to optimize the sequencing results.

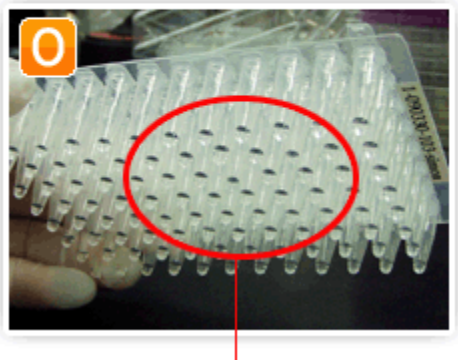
⚠ Please place your samples properly into strip-capped well plate as shown below.



⚠ To avoid any physical damage, please use out-skirted well plate.

Out-skirted well plate is Good!!

⚠ Please seal tightly to avoid any potential damages in transit such as evaporation or contamination of samples during shipping.



Enough volume and constant concentration at each well is the key factor for good results!

Bad sealing and leaking may lead cross-contamination and unsatisfactory results!

Macrogen Supplied Universal Primers

Name	Sequence (5'-3')	Name	Sequence (5'-3')
1492R	TACGGYTACCTTGTTACGACTT	M13R-pUC	CAGGAAACAGCTATGAC
27F	AGAGTTTGATCMTGGCTCAG	MT_Forward	CATCTCAGTGCAACTAAA
35S-A	AAGGGTCTTGCGAAGGATAG	pBacPAC-RP	GTCTGTAAATCAACAACGC
35S-B	AGTGGAAAAGGAAGGTGGCT	pBAD-F	ATGCCATAGCATTTTTATCCA
518F	CCAGCAGCCGCGGTAATACG	pBAD-FP	ATGCCATAGCATTTTTATCC
800R	TACCAGGGTATCTAATCC	pBAD-R	GATTTAATCTGTATCAGG
AD_Reverse	AGATGGTGCACGATGCACAG	pDONOR-FP	TAACGCTAGCATGGATCTC
a-Factor	TACTATTGCCAGCATTGCTGC	pEGFP_N	CCGTCCAGCTCGACCAG
AOX1_Forward	GACTGGTTCCAATTGACAAGC	pEGFP-FP	TTTAGTGAACCGTCAGATC
AOX1_Reverse	GCAAATGGCATTCTGACATCC	pEGFP-RP	AACAGCTCCTCGCCCTTG
BGH-R	TAGAAGGCACAGTCGAGG	pESP-RP	TCCAAAAGAAGTCGAGTGG
Bluescript_KS	TCGAGGTCGACGGTATC	pET-24a	GGGTTATGCTAGTTATTGCTCAG
Bluescript_SK	CGCTCTAGAACTAGTGGATC	pET-RP	CTAGTTATTGCTCAGCGG
CMV-F	CGCAAATGGGCGGTAGGCGTG	pFastBac_Forward	GGATTATTCATACCGTCCCA
CYC1_Reverse	GCGTGAATGTAAGCGTGAC	pFastBac_Reverse	CAAATGTGGTATGGCTGATT
DsRed1-C	AGCTGGACATCACCTCCCACAACG	pGEX3	GGAGCTGCATGTGTCAGAGG
DsRed1-N	GTACTGGAACTGGGGGGACAG	pGEX5	GGCAAGCCACGTTTGGTG
EBV-RP	GTGGTTTGTCCAAACTCATC	pJET1_2F	CGACTCACTATAGGGAGAGCGGC
EGFP-C	CATGGTCCTGCTGGAGTTCGTG	pJET1_2R	AAGAACATCGATTTTCCATGGCAG
EGFP-CF	AGCACCCAGTCCGCCCTGAGC	pMalE	TCAGACTGTCGATGAAGC
EGFP-CR	CGTCCATGCCGAGAGTG	pQE-F	CCCGAAAAGTGCCACCTG
EGFP-N	CGTCGCCGTCCAGCTCGACCAG	pQE-R	GTTCTGAGGTCATTACTGG
EGFP-NR	CGTCGCCGTCCAGCTC	pREP-fwd_primer	GCTCGATACAATAAACGCC
GAL1_Forward	AATATACCTCTATACTTTAACGTC	pRH_Forward	CTGTCTCTATACTCCCCTATAG
Gal4AD	TACCACTACAATGGATG	pRH_Reverse	CAAAATTCAATAGTTACTATCGC
GLprimer1	TGTATCTTATGGTACTGTAACTG	pTrcHis_Forward	GAGGTATATATTAATGTATCG
GLprimer2	CTTTATGTTTTTGGCGTCTTCCA	QE_Promoter	CCGAAAAGTGCCACCTG

HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	RVprimer3	CTAGCAAAATAGGCTGTCCC
ITS1	TCCGTAGGTGAACCTGCGG	RVprimer4	GACGATAGTCATGCCCCGCG
ITS2	GCTGCGTTCTTCATCGATGC	SP6	ATTTAGGTGACACTATAG
ITS3	GCATCGATGAAGAACGCAGC	STag_18mer_Primer	GAACGCCAGCACATGGAC
ITS4	TCCTCCGCTTATTGATATGC	SV40-pArev	CCTCTACAAATGTGGTATGG
ITS5	GGAAGTAAAAGTCGTAACAAGG	SV40-Promoter	GCCCCTAACTCCGCCCATCC
KAN2-FP	ACCTACAACAAAGCTCTCATCAACC	T3	ATTAACCCTCACTAAAG
KAN2-RP	GCAATGTAACATCAGAGATTTTGAG	T7	AATACGACTCACTATAG
LCO1490	GGTCAACAAATCATAAAGATATTGG	T7_EEV	ATGTCGTAATAACCCCGCCCCG
M13F	GTAAAACGACGGCCAGT	T7promoter	TAATACGACTCACTATAGGG
M13-FP	TGTAAAACGACGGCCAGT	T7terminator	GCTAGTTATTGCTCAGCGG
M13F-pUC	GTTTTCCCAGTCACGAC	U-19mer_Primer	GTTTTCCCAGTCACGACGT
M13R	GCGGATAACAATTTCACACAGG		