Macrogen Sample Submission Guide

Macrogen has served over 10 years in sequencing field using the cutting edge technology and delivering fast and reliable results. We use high throughput Applied Biosystems 3730XL sequencers. We sequence all kinds of cloned DNAs (plasmids, cosmid, phages, BACs) as well as PCR-products. Macrogen provides a high quality DNA Sequencing service for academics, government, research institutions and private companies at **Very Competitive Price**.
Please contact us for any inquiry.

For more detailed explanations and recommendations about the preparation and submission of samples, please refer to the following link:
http://dna.macrogen.com/eng/help/sample_submission.jsp

For information regarding shipping specifications, please refer to the following link:
http://dna.macrogen.com/eng/help/orderg.jsp

To order the sequencing service, please access to our online ordering system and create an account;
http://dna.macrogen.com/eng

For payment information, please refer to the following link:
http://dna.macrogen.com/eng/help/howtopay.jsp

Contact

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Payment Inquiry: payment@macrogen.com

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Template Type/Format	Sample Requirements	Remarks
Plasmid	* 100 ng/µl * Minimum volume of 20µl	For re-sequencing, at lease 5 µl is required.
16S	* Agar Plate/Glycerol Stock * gDNA:30-50ng * Minimum volume of 20µl	N/A
PCR Product (Purified)	* 50 ng/µl * Minimum volume of 20µl	For re-sequencing, at lease 5 µl is required.
PCR Product (Unpurified)	* 100 ng/µl * Minimum volume of 30µl	N/A
Difficult Sequence	* 100 ng/µl * Minimum volume of 40µl	N/A
Primer Walking	* 8 µg (for an insert size of up to 4kb)	Single Strand Sequencing: 1 µg/1 kb insert. If insert size is longer than 4kb, clone is required in an agar stab culture status.

It is possible to send us either a stab or glycerol culture with the selected *E.coli* clone, the purified plasmid DNA or your unpurified or purified PCR product.
Templates and primers must be provided in DI water or 10mM Tris buffer, not in TE

a) Sample for individual tube :

- General glycerol stock/ Agar-stab/Agar-plate culture at room temperature.
- 1.5µl microcentrifuge tube is recommended in a dried (lyophilized) form or solution (Nuclease-free TE or distilled water) at room temperature..
- Free re-sequencing is included

b) 96 Well Plate :

- 8 strip cap is recommended in a dried (lyophilized) form or solution (Nuclease-free TE or distilled water) at room temperature.

* Note: The well to well variation of the concentration of your samples is very important to get better results, please keep it as constant as possible.

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

Strip cap is Good!

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

Out-skirted well plate is Good!

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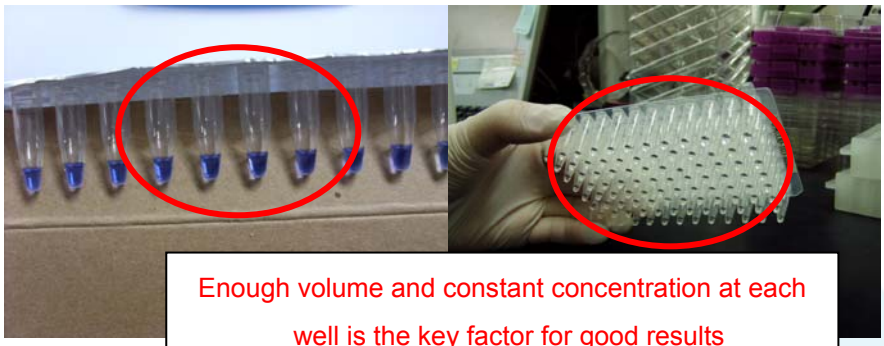
Please seal tightly to avoid any potential damages in transit such as evaporation or contamination of samples during shipping.



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!

- Re-sequencing for plate is additionally charged.

** In case of Multi primers for one plate **

Only two custom primers (or two universal primers) in tubes could be acceptable for applying this Plate sequencing.

* Note : For three or more primers, another paired primer plate with your sample plate should be provided. If not, your order will be regarded as Normal sequencing reactions.

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Template Preparation Guidelines

1) Template preparation

The success of automated sequencing critically depends on having high purity template in the correct concentration. In case of samples in plates, constant concentration among wells is also important for the success of sequencings.

i) Plasmid DNA

Preparation

There are many commercial kits available. Please submit DNA in deionized water. **Do not** use TE to dilute or re-suspend the DNA because EDTA inhibits the cycle of sequencing reaction We recommend using Qiagen miniprep/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 2 µg. 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.

ii) PCR fragments

Preparation

The DNA is 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.

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2) Primers preparation

Primer Considerations

Primers should be provided in DI water at the required concentration (see table above).

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

Please supply primers at concentration of (10 pmole/µl =60 ng/µl) in deionized water at volume of greater than 20 µl.

Primers supplied by customers should be desalted or purified. Crude primers generally do not work well for sequencing. We have the following primers available at no extra charge.

Primer Name	Sequence	Base
T7	AATACGACTCACTATAG	17
T7terminator	GCTAGTTATTGCTCAGCGG	19
T7promoter	TAATACGACTCACTATAGGG	20
T3	ATTAACCCTCACTAAAG	17
SP6	ATTTAGGTGACACTATAG	18
M13F-pUC(-40)	GTTTTCCCAGTCACGAC	17
M13R-pUC(-40)	CAGGAAACAGCTATGAC	17
M13F(-20)	GTAAAACGACGGCCAGT	17
M13R(-20)	GCGGATAACAATTTCACACAGG	22
pGEX5	GGCAAGCCACGTTTGGTG	18
pGEX3	GGAGCTGCATGTGTCAGAGG	20
27F	AGAGTTTGATCMTGGCTCAG	20
1492R	TACGGYTACCTTGTTACGACTT	22
518F	CCAGCAGCCGCGGTAATACG	20
800R	TACCAGGGTATCTAATCC	18
ITS1	TCCGTAGGTGAACCTGCGG	19

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ITS4	TCCTCCGCTTATTGATATGC	20
BGH-R	TAGAAGGCACAGTCGAGG	18
CMV-F	CGCAAATGGGCGGTAGGCGTG	21
RVprimer3	CTAGCAAAATAGGCTGTCCC	20
RVprimer4	GACGATAGTCATGCCCCGCG	20
GLprimer1	TGTATCTTATGGTACTGTAACTG	23
GLprimer2	CTTTATGTTTTTGGCGTCTTCCA	23
pQE-F	CCCGAAAAGTGCCACCTG	18
pQE-R	GTTCTGAGGTCATTACTGG	19
Gal4AD	TACCACTACAATGGATG	17
pBAD-F	ATGCCATAGCATTTTTATCCA	21
pBAD-R	GATTTAATCTGTATCAGG	18
EGFP-CF	AGCACCCAGTCCGCCCTGAGC	21
EGFP-CR	CGTCCATGCCGAGAGTG	17
EGFP-NR	CGTCGCCGTCCAGCTC	16

3) 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 but JM101 is often poor.

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

4) 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 good way to quantitate DNA is to run an aliquot on a minigel 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 10 ng/100 bases, and at least 20 ng/µl solution in deionized water. Please provide at least 10 ul for any possible re-sequencing.

Please be advised that "Gel Electrophoresis rather than Nano-drop "is recommended