Macrogen Single Pass Sequencing Sample Submission Guide

Macrogen has served over 10 years in sequencing field using the cutting edge technology and de livering fast and reliable results. We use high throughput Applied Biosystems 3730XL sequence rs. We sequence all kinds of cloned DNAs (plasmids, cosmid, phages, BACs) as well as PCR-pr oducts. 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.

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

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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 p lasmid 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.

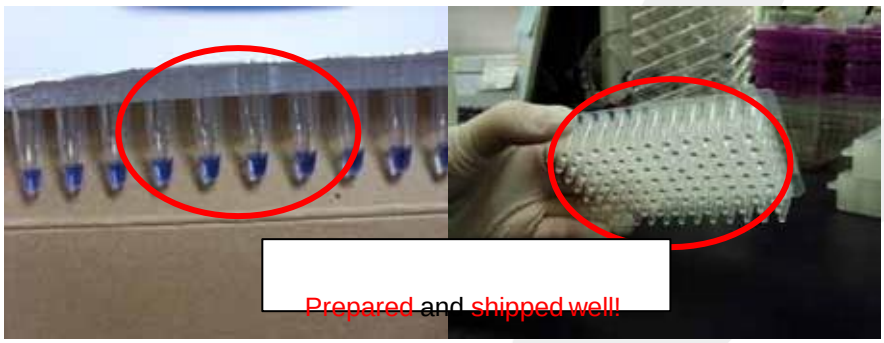
Please place your samples properly into strip- capped well plate as shown below.

Strip cap is Good!

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

Out-skirted well plate is Good!

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



Prepared and shipped well!

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Bad sealing may lead cross-contamination and unsatisfactory results!

-Re-sequencing is additionally charged.

-Please prepare samples to avoid **any well-to-well concentration difference** or **size difference** for quality results.

Template Preparation Guidelines

1) Template preparation

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

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 reco mmend using Qiagen miniprep/midiprep since both methods yield consistent purity of plasmid D NA 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.

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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.

2) 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.

3) 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 deio nized water. Please provide at least 10 ul for any possible re-sequencing.

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

4) 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(5'->3')	Base
Bluescript SK	CGCTCTAGAACTAGTGGATC	20
EBV-RP	GTGGTTTGTCCAAACTCATC	20
KAN2-FP	ACCTACAACAAAGCTCTCATCAACC	25
KAN2-RP	GCAATGTAACATCAGAGATTTTGAG	25
M13-FP	TGTAAAACGACGGCCAGT	18
pBacPAC-RP	GTCTGTAAATCAACAACGC	19
pBAD-FP	ATGCCATAGCATTTTTATCC	20
pDONOR-FP	TAACGCTAGCATGGATCTC	19
pEGFP_N	CCGTCCAGCTCGACCAG	17
pEGFP-FP	TTTAGTGAACCGTCAGATC	19
pEGFP-RP	AACAGCTCCTCGCCCTTG	18
pESP-RP	TCCAAAAGAAGTCGAGTGG	19
pET-24a	GGGTTATGCTAGTTATTGCTCAG	23
pET-RP	CTAGTTATTGCTCAGCGG	18
pMalE	TCAGACTGTCGATGAAGC	18
pREP-fwd	GCTCGATACAATAAACGCC	19
35S-A	AAGGGTCTTGCGAAGGATAG	20
35S-B	AGTGGAAAAGGAAGGTGGCT	20
AD Reverse	AGATGGTGCACGATGCACAG	20
CYC1 Reverse	GCGTGAATGTAAGCGTGAC	19
DsRed1-C	AGCTGGACATCACCTCCCACAACG	24
DsRed1-N	GTACTGGAACTGGGGGGACAG	21
EGFP-C	CATGGTCCTGCTGGAGTTCGTG	22
EGFP-N	CGTCGCCGTCCAGCTCGACCAG	22
GAL1 Forward	AATATACCTCTATACTTTAACGTC	24
U-19mer Primer	GTTTTCCCAGTCACGACGT	19
T7 EEV	ATGTCGTAATAACCCCGCCCCG	22
Bluescript KS	TCGAGGTCGACGGTATC	17
pFastBac Forward	GGATTATTCATACCGTCCCA	20
pFastBac Reverse	CAAATGTGGTATGGCTGATT	20
AOX1 Forward	GACTGGTTCCAATTGACAAGC	21
AOX1 Reverse	GCAAATGGCATTCTGACATCC	21

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a-Factor	TACTATTGCCAGCATTGCTGC	21
STag 18mer Primer	GAACGCCAGCACATGGAC	18
MT Forward	CATCTCAGTGCAACTAAA	18
QE Promoter	CCGAAAAGTGCCACCTG	17
pRH Forward	CTGTCTCTATACTCCCCTATAG	22
pRH Reverse	CAAAATTCAATAGTTACTATCGC	23
SV40-pArev	CCTCTACAAATGTGGTATGG	20
SV40-Promoter	GCCCCTAACTCCGCCCATCC	20
pTrcHis Forward	GAGGTATATATTAATGTATCG	21
ITS1	TCCGTAGGTGAACCTGCGG	19
ITS2	GCTGCGTTCTTCATCGATGC	20
ITS3	GCATCGATGAAGAACGCAGC	20
ITS4	TCCTCCGCTTATTGATATGC	20
pJET1.2R	AAGAACATCGATTTTCCATGGCAG	24
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
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

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