

Macrogen Single Pass Sequencing 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>

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| Template Type/Format | Sample Requirements | Remarks |
|--------------------------|---|---|
| Plasmid | * 100 ng/μl * Minimum volume of 20μl | For re-sequencing, at least 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 least 5 μl is required. |
| PCR Product (Unpurified) | * 100 ng/μl * Minimum volume of 20μl | 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 :

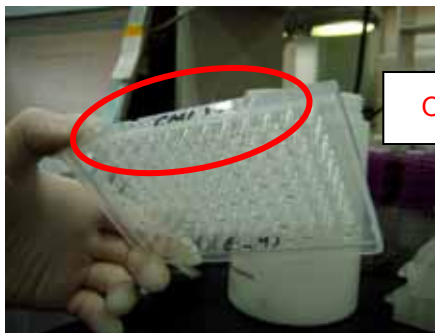
- 8 strip cap is recommended in a dried (lyophilized) form or solution (Nuclease-free TE or distilled water) at room temperature.

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!



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 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.

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/ μ l solution in deionized water. Please provide at least 10 μ l for any possible re-sequencing.

Measurement with electrophoresis is recommended rather than spectrophotometer-based measurements.

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 T_m (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/ μ l =60 ng/ μ l) in deionized water at volume of greater than 20 μ l.

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 | TGTA AACGACGGCCAGT | 18 |
| pBacPAC-RP | GTCTGTAAATCAACAACGC | 19 |
| pBAD-FP | ATGCCATAGCATT TTTTATCC | 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 | TCAGACTGTGATGAAGC | 18 |
| pREP-fwd | GCTCGATAACAATAAACGCC | 19 |
| 35S-A | AAGGGTCTTGCGAAGGATAG | 20 |
| 35S-B | AGTGGA AAAAGGAAGGTGGCT | 20 |
| AD Reverse | AGATGGTGCACGATGCACAG | 20 |
| CYC1 Reverse | GCGTGAATGTAAGCGTGAC | 19 |
| DsRed1-C | AGCTGGACATCACCTCCCACAACG | 24 |
| DsRed1-N | GTA CTGGA ACTGGGGGGACAG | 21 |
| EGFP-C | CATGGTCCTGCTGGAGTTCGTG | 22 |
| EGFP-N | CGTCGCCGTC CAGCTCGACCAG | 22 |
| GAL1 Forward | AATATACCTCTATACTTTAACGTC | 24 |
| U-19mer Primer | GTTTTCC CAGTCACGACGT | 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 |

| | | |
|--------------------|--------------------------|----|
| a-Factor | TACTATTGCCAGCATTGCTGC | 21 |
| S-Tag 18mer Primer | GAACGCCAGCACATGGAC | 18 |
| MT Forward | CATCTCAGTGCAACTAAA | 18 |
| QE Promoter | CCGAAAAGTGCCACCTG | 17 |
| pRH Forward | CTGTCTCTATACTCCCCTATAG | 22 |
| pRH Reverse | CAAATTC AATAGTTACTATCGC | 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) | GTTTTCCAGTCACGAC | 17 |
| M13R-pUC(-40) | CAGGAAACAGCTATGAC | 17 |
| M13F(-20) | GTAAAACGACGGCCAGT | 17 |
| M13R(-20) | GCGGATAACAATTTACACAGG | 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 | CTAGCAAATAGGCTGTCCC | 20 |
| RVprimer4 | GACGATAGTCATGCCCCGCG | 20 |
| GLprimer1 | TGTATCTTATGGTACTGTA ACTG | 23 |
| GLprimer2 | CTTTATGTTTTTGCGTCTTCCA | 23 |
| pQE-F | CCCGAAAAGTGCCACCTG | 18 |
| pQE-R | GTTCTGAGGTCATTACTGG | 19 |
| Gal4AD | TACCACTACAATGGATG | 17 |

| | | |
|----------|-------------------------|----|
| pBAD-F | ATGCCATAGCATT TTTTATCCA | 21 |
| pBAD-R | GATTTAATCTGTATCAGG | 18 |
| EGFP-CF | AGCACCCAGTCCGCCCTGAGC | 21 |
| EGFP-CR | CGTCCATGCCGAGAGTG | 17 |
| EGFP-NR | CGTCGCCGTCCAGCTC | 16 |
| pJET1.2F | CRACTCACTATAGGGAGAGCGGC | 23 |

