

Materials required for the services

I. Template:

A. Quality:

Template quality is the most important factor for successful sequencing. Trace amount of protein, RNA, salt, carbohydrate, organic solvent and alcohol, EDTA as in TE can contribute to the failure of sequencing. We recommend the following guidelines for preparing sequencing quality template. For additional charge, we can prepare sequencing quality template from bacterial stock.

1) Plasmid DNA:

- i. DNA template should be prepared by either CsCl or Qiagen's (or compatible) column purification method.
- ii. DNA template should have a O.D. ratio of 1.8 – 2.0 between 260 and 280 nm.
- iii. Ideally, DNA should be resuspended in water. 10 mM Tris is acceptable. Avoid TE.
- iv. If organic solvent is used such as phenol and chloroform in the purification process, the DNA should be purified by at least two times of ethanol precipitation to remove trace amount of organic solvent.
- v. If ethanol or isopropanol is used to precipitate DNA, perform the precipitation using ammonium acetate instead of sodium acetate. Wash the DNA pellet after centrifugation at least once with cold 70% ethanol. Then, dry the DNA pellet as much as possible before resuspending it in water. Trace amount of leftover alcohol is one of the major causes of DNA sequencing failure.
- vi. If mini spin column is used to purify DNA, we recommend spin the column twice for 5 min. after the washing step. Then, elute the DNA from column with water. This will eliminate leftover alcohol from wash buffer in the sample.

2) PCR products:

- i. PCR product should be a single band in the gel. (We DO NOT accept PCR fragment purified from PCR with multiple bands because the successful rate of getting good sequence is very low).
- ii. PCR products should be at least 150 bp. Smaller PCR products are very difficult to sequence as they work as primers after denaturation and are difficult to purify with good yields with spin column based kit.
- iii. PCR product should be gel purified using Qiagen's (or compatible) gel purification kit. We recommend a gel of at least 2% for purification. PCR products purification kit is often not good enough to prepare sequencing quality template.
- iv. Ideally, gel purified PCR products should be resuspended in water. 10 mM Tris is acceptable. Avoid TE.
- v. If mini spin column based gel purification kit is used to purify PCR product, we recommend spin the column twice for 5 min. after the washing step. Then, elute the DNA from column with water. This will eliminate leftover alcohol from wash buffer in the sample.

B. Quantity:

Accurate quantitation of the DNA template is important for successful sequencing. Not enough template will produce data with low signal strengths, short sequences, noisy data, and ambiguities. Too much template will show data with trailing peaks and may be accompanied by weaker signal.

1) Plasmid DNA:

Basic and Express sequencing services

2 µg of DNA at concentration of at least 0.2 µg/µl. If your DNA concentration is lower than 0.2 µg/µl, we can concentrate the sample for you.

Confirmation, single-strand, double-strand accuracy sequencing services

2 µg per kilobase of DNA at concentration of at least 0.2 µg/µl.



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2) PCR products:**One-Reaction sequencing**

- 0.2 µg at concentration of at least 0.1 µg/µl for PCR products less than 1 kb.
- 0.2 µg per kilobase of DNA at concentration of at least 0.2 µg/µl.
- If the PCR product is larger than 3 kb, we recommend cloning it into a TA vector and follow the guidelines for sequencing plasmid DNA.

II. Primer:**A. Design guideline:**

- 1) Make sure the sequence used to design primer is accurate.
- 2) The length of the primer should be at least 18 bases in length with optimal being 20-25 bases.
- 3) The G-C content of the primer should be between 40-60% with optimal being 50%.
- 4) Ideally, the four bases, A,T, G,C should be distributed in the primer as evenly as possible.
- 5) The T_m of the primer should be between 55 and 70 °C. Use the following formula to calculate the T_m. $T_m = 69.3 + 0.41 \times (\%GC) - 650/\text{length of primer}$
- 6) Having G or C on 3' and 5' end and avoid T.
- 7) Avoid long stretches of a single base (more than 3).
- 8) Design the primer at least 50 bases upstream of the target sequence. Sequence data is often more accurate after 80 bases downstream of the primer.
- 9) Avoid sequence that has a potential to self-anneal and form secondary structure.

B. Amount:

10 µl at concentration of at least 10 µM in water.

C. Common primers provided at no additional charge:

M13/pUC forward primer (5' GTAAAACGACGGCCAGTG 3')

M13/pUC reverse primer (5' CAGGAAACAGCTATGACC 3')

T7 promoter primer (5' TAATACGACTCACTATAGGG 3')

T7 terminator primer (5' TATGCTAGTTATTGCTCAGC 3')

T3 promoter primer (5' AATTAACCCTCACTAAAGGG 3')

Sp6 promoter primer (5' CATACGATTTAGGTGACACTATAG 3')

CMV forward primer (5' CGCAAATGGGCGGTAGGCGTG 3')

BGH reverse primer (5' TAGAAGGCACAGTCGAGG 3')

Materials will be emailed to the contracting laboratory

- Basic Sequencing service provides three files by email: 1) Sequence in a text format (.txt), 2) Chromatograph in a PDF format (.pdf), 3) Chromatograph in AB1 format (AB1).
- Project-based DNA sequencing services include 1) Individual chromatograms of each sequencing run, 2) Report with the final assembled sequence, 3) Leftover primers.



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Time required for the service**I. One-reaction DNA sequencing services**

2 - 3 working days for simple projects.

II. Project-based DNA sequencing services

Confirmation and Single-strand accuracy: 1-3 kb per week

Double-strand accuracy: 0.5-2 kb per week

Additional time is required for large project and primer synthesis.

Time requirement is estimated based on a single project and assuming each run produces good quality sequence. Additional time may be needed depending on the nature of the project and the quality of each sequencing run. Extra time also needed for plasmid and PCR product purification.

Ordering Information *

| | | |
|--------|---|---------|
| MS800B | Basic DNA sequencing service | \$ 20 |
| MS800E | Express DNA sequencing service | \$ 12 |
| MS801 | Sequencing grade primer synthesis | \$ 1.10 |
| MS802 | Basic DNA sequencing plus mini-scale DNA purification (DNA to be sequence is provided to us in the form of bacteria containing plasmid less than 20 kb) | \$ 40 |
| MS802M | Basic DNA sequencing plus 250-ml scale DNA purification (DNA to be sequence is provided to us in the form of bacteria containing plasmid less than 20 kb) | \$ 120 |
| MS805C | Sequencing project – Confirmation accuracy | \$ 0.20 |
| MS805D | Sequencing project – Double-strand accuracy | \$ 0.45 |
| MS805S | Sequencing project – Single-strand accuracy | \$ 0.28 |
| MS809 | Additional reagents charge | Inquire |

*Government contract, academic discount, volume discount are available. Please contact us for a quote

Related Services

Plasmid DNA purification services

For more information, contact us at 301-831-1377 or info@mtrscientific.com



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