

# DNA Sequencing Services ver. 2009

## Services description

Sequencing reactions are performed using the ABI BigDye chemistry and analyzed on ABI 3100 Genetic Analyzer. On an average, each good sequencing run will give 600-900 bases of sequences. Among them 500-700 bases can be read and used with certainty (Readable sequences). Within the readable sequence, 400-600 bases are in the region represents by strong, single peaks (Quality sequences).

### **I. Standard One-Reaction DNA Sequencing Services:**

#### **1. Basic DNA Sequencing Services.**

This DNA sequencing service is designed to meet the demand for sequence with high accuracy. It uses enhanced protocol with proprietary sequencing mix for longer, more accurate sequence than standard techniques. In addition to a 500 bp readable sequence guarantee\*, the service includes manual sequence editing to correct computer error (extends sequence accuracy to 800 bases or more), helpful comments on quality of the sequence, no-charge DNA concentration for low yield plasmid, gel filtration purification for low quality DNA and a no charge, one-time repeat if sequencing problem caused by low concentration of template.

#### **2. Express DNA Sequencing Services.**

This low cost, fast turnaround service uses standard sequencing protocol. It has a 500 bases readable sequence guarantee\* if the template and primer provided are of sequencing quality. This service provides sequence without manual editing and other added values included in our Basic DNA sequencing service.

\* 500 bases readable sequence guarantee applies to most sequencing quality DNA templates with the exception of DNA templates containing G-C rich sequences and secondary structures that cannot be resolved by standard sequencing techniques.

### **II. Project-based DNA Sequencing Services:**

These services include primer design, synthesis, and sequence assembly.

#### **1. Confirmation accuracy:**

This service is recommended for sequencing project in which the sequence of the target DNA is known. Since we can use the known sequence as roadmap, some of the sequences adjacent to the good quality sequences can still be usable. Therefore, we can extend the usable sequence at each run to 450-650 bases. With this trade-off on accuracy, the cost of the sequencing project will be significantly reduced.

#### **2. Single-strand accuracy:**

Only good quality sequence from each run will be used for the final sequence assembly. Each base in the target DNA will be sequenced at least once from either sense or antisense direction.

#### **3. Double-strand accuracy:**

Only good quality sequence from each run will be used for the final sequence assembly. Each base in the target DNA will be sequenced at least once from both the sense and the antisense direction.



**MTR SCIENTIFIC**  
9639-122 Dr. Perry Rd., Ijamsville, MD 21754  
Phone: 301-831-1377 Fax: 301-874-1899  
<http://www.mtrscientific.com>  
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### Difference between Express and Basic DNA Sequencing Services

	Express	Basic
Typical # of bases provided	600-800 bases	700-950 bases
Sequencing protocol	Standard protocol and standard sequencing reaction mix	Enhanced protocol with proprietary sequencing mix for longer, more accurate sequence
Typical turnaround time	2 - 3 days	3 days
Results will be provided by email	Yes	Yes
500 bp readable sequence guarantee*	Yes	Yes
Manual editing to extend accuracy	No	Yes
Typical accuracy	500-600 bases	700-900 bases
Chromatogram provided in ab1 format	Yes	Yes
Conversion of chromatogram to pdf format for easy viewing	Yes	Yes
Sequence provided in text file format	Yes	Yes
Helpful comments on sequencing results provided	No	Yes
DNA concentration if sample is too diluted	Additional Charge	Free
Gel filtration purification if DNA is in TE (TE inhibits sequencing reaction)	Additional Charge	Free
No charge, one-time repeat if sequencing problem caused by low concentration of template	No**	Yes
List price***	\$12	\$20

\* 500 bp readable sequence guarantee applies to most sequencing with quality DNA templates and primers with the exception of DNA templates containing G-C rich sequences and secondary structures that cannot be resolved by standard sequencing techniques.

\*\* For express grade service, we will repeat sequencing on request. If the quality of repeated sequence is similar to the initial sequence, we will charge the second run. If the quality of the repeated sequence is better the initial sequence, we will not charge the repeat.

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



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## 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<sub>m</sub> of the primer should be between 55 and 70 °C. Use the following formula to calculate the T<sub>m</sub>.  $T_m = 69.3 + 0.41 \times (\%GC) - 650/\text{length of primer}$
- 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](mailto:info@mtrscientific.com)**



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**DNA Sequencing Services Request Form**

Date: \_\_\_\_\_ P.O. Number: \_\_\_\_\_

Investigator Name: \_\_\_\_\_ Phone: \_\_\_\_\_ Email: \_\_\_\_\_

Address: \_\_\_\_\_

**DNA Sequencing Service**

- Basic  Express  
 Confirmation Accuracy  Single-strand accuracy  Double-strand accuracy

**Template information:**

Plasmid Name: \_\_\_\_\_ Plasmid size: \_\_\_\_\_

Vector Name: \_\_\_\_\_ Vector size: \_\_\_\_\_

Insert/ PCR Fragment Name: \_\_\_\_\_ Insert/PCR Fragment Size: \_\_\_\_\_

- Additional plasmid/ DNA fragment information attached

**Template provided as**

- Purified PCR reaction

Concentration/Total volume (0.2 µg at concentration of at least 0.1 µg/µl for good results): \_\_\_\_\_

- DNA Concentration/Total volume (2 µg at concentration of at least 0.2 µg/µl for good results): \_\_\_\_\_  
 Prepared by  CsCl  Column method  Other \_\_\_\_\_

Preparation method  Mini-prep  Midi-Prep  Maxi-prep  
 DNA in (Avoid TE)  water (preferred)  10 mM Tris buffer  Others \_\_\_\_\_Bacteria (for purification services) as  glycerol stock,  colony on plate,  liquid culture  
 Host strain: \_\_\_\_\_ Selection Antibiotic \_\_\_\_\_, Concentration \_\_\_\_\_Plasmid Purification Services:  Sequencing only  200-ml scale

- Large scale (please also complete plasmid service request form)

- Primer:**  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 ReversePrimer (5' TAGAAGGCACAGTCGAGG 3')  
 Oligo synthesis service (please complete our service form on page 6)  
 Primer Provided (Require at least 5 µl at concentration of at least 10 µM per reaction)

Name of primer: \_\_\_\_\_ Concentration: \_\_\_\_\_ Volume: \_\_\_\_\_

Sequence (optional): \_\_\_\_\_

Additional Request: \_\_\_\_\_



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