Service Types

Sanger Sequencing

Decision #1: Standard vs. Difficult Template

A. Standard Template

Most plasmid samples would be considered standard. Our Sanger sequencing SOP (standard operating procedure) is designed to work with miniprep-quality recombinant plasmid DNA and a high-specificity sequencing primer to give contiguous reads >800 bases and over 1000 high-quality (Q>20) Phred calls

B. Difficult Template

We will add our proprietary Difficult Template Buffer (DTB) to your sample to facilitate template denaturation and processivity of the AmpliTaq DNA polymerase during cycle sequencing. Common reasons for processivity problems are:

- GC-rich templates, with overall greater than 60-65% GC content
- GC-rich regions, with greater than 60-65% GC content (minimum 100-150 bp)
- Homopolymers [such as poly(A) tails]
- Repetitive sequences (di- or tri-nucleotide repeats, such as His tags)

Decision #2: My Primer vs. Core Primer

A. My Primer

You supply a primer for cycle sequencing. **YOU MUST ADD THE PRIMER TO YOUR SAMPLE** (aka pre-mixed).

We reserve the right to delay or refuse orders with the primer supplied separately.

B. Core Primer

We can supply any of these 10 common MCS primers for cycle sequencing:

- T3 Promoter
- T7 Promoter
- T7 Terminator
- pGEX 5’
- pGEX 3’
- SP6 Promoter
- M13 Forward (-20)
- M13 Forward (-41)
- M13 Reverse (-27)
- M13 Reverse (-48)

- 5'-AA TTA ACC CTC ACT AAA GGG-3'
- 5'-TAA TAC GAC TCA CTA TAG GG-3'
- 5'-GCT AGT TAT TGC TCA GCG G-3'
- 5'-GGG CTG GCA AGC CAC GTT TGG TG-3'
- 5'-CCG GGA GCT GCA TGT GTC AGA GG-3'

- 5'-TAC GAT TTA GGT GAC ACT ATA G-3'
- 5'-GTA AAA CGA CGG CCA GT-3'
- 5'-CGC CAG GGT TTT CCC AGT CAC GAC-3'
- 5'-CAG GAA ACA GCT ATG AC-3'
- 5'-AGC GGA TAA CAA TTT CAC ACA GG-3'

Decision #3: Tubes vs. 96-Well Plate

A. Tubes

- For < 24 samples only, preferably
- Please use 1.5 mL tubes
- See Sample Requirements for more details

B. 96-Well Plate

- Preferred for 24-47 samples
- Required for 48+ samples (includes price break)
- Please use PCR plates with conical wells
- Put your samples in order **vertically**, starting with well A1 (sample 1=A1, sample 2=B2, etc.)
- See Sample Requirements for more details

For 24-47 samples, we **CAN'T** guarantee same day service for individual samples because it slows down our workflow.

PCR Cleanup + Sanger Sequencing

PLEASE NOTE: We offer a "one-size-fits-all" protocol for PCR cleanup with no quantification. Thus, we highly recommend that you clean your PCR product and thoroughly analyze it via gel electrophoresis and fluorometry prior to Sanger sequencing. Recommended concentrations can be found in Template Prep.

Custom Sequencing

Although rare, sometimes DTB and/or our standard Sanger protocol are not sufficient for sequencing a DNA template. We offer custom sequencing for these very difficult templates, which generally involves alternative sequencing chemistry or other modifications to our SOP. We are happy to consult with you regarding custom sequencing at no charge. However, we do charge a higher fee for custom sequencing to cover the cost of the specialized reagents (currently $5.75/sample for UT). Examples:

- Problematic secondary structure (such as hairpin loops, stem loops, or palindromic sequences)
- Sequencing directly from gDNA

- PCR amplicons <200 bp or vectors larger than 15 Kb