Sanger-based DNA Sequencing - Frequently Asked Questions

Why did my template not sequence?

The two most critical factors for the success of automated sequencing are DNA sample purity and concentration. The most common cause for reaction failure or poor quality sequencing results in our facility is DNA concentration. That's good to know because it is easy to fix! Right?

The DNA needs to come in at 100 ng/µl. We highly recommend that customers use either the QIAGEN plasmid DNA prep kits to purify their plasmid DNA samples or the Promega SV plasmid isolation kits.

Why should I resuspend my template in water?

The presence of salts or the EDTA in TE buffer will result in sequencing reaction failure.

EDTA is known to chelate magnesium and limit the activity of Taq DNA polymerase. We recommend that all templates be reconstituted in water. If you feel strongly about buffering your DNA you may store DNA in 10 mM Tris.

Does the host strain matter?

There are strains of *E. coli* that are better for plasmid production. Recommended strains are XL1-Blue, DH5(alpha), DH1, C600 SURE, NM294. Other strains such as JM 100 series, NM522, NM544, TB1, TG1, BL21, MC1061 do not produce good sequence quality DNA. These strains contain large amounts of carbohydrate that are released during cell lysis, and also contain an Endo A locus which results in the production of large amounts ofnuclease.

Does it matter which media I use to grow my cells?

Do not overgrow *E. coli* cells, especially in simple LB, as the cells decline rapidly after reaching stationary phase. Overgrowth can result in the early cell lysis, leading to the release of degraded chromosomal DNA and reduced plasmid quality. Standard LB with antibiotic selection is recommended for most protocols, with incubation of no longer than 16 hours to prevent cells from lysis.

My sequence stopped short. Why?

Please look at the sequence preceding termination.

a) Is there a Poly A and/or T region? Frequently the polymerase will slip while trying to navigate these monomer repeats. This will result in the sequence becoming noisy on the other side of the repeat. The SMF has a poly T and a poly A primer to get through these regions.

b) Short tandem repeats, e.g. CTCTCTCTCTCTCTC, can cause the polymerase to terminate prematurely. The facility has several methods to try and navigate difficult regions of sequence. (Please talk to Erika if you have this kind of request.)

c) High GC content. This will frequently cause sequencing reactions to terminate due to hairpin loop formation within a sequence. The facility has a number of ways to navigate this kind of template depending on the sequence.