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Frequently Asked Questions DNA Sequencing Page 1

Q- How much DNA do I submit? At what concentration?

We ask you to submit **25 µl** of sample in distilled water or 1X Tris at the following concentrations:

For Plasmid DNA:

50 ng/µl or greater.

For PCR Product:

10 ng/µl or greater.

For BAC DNA:

250 ng/µl or greater, or we can do the DNA prep.

Remember, the higher the concentration, the better. With higher concentrations, we can make dilutions of the template, which will also dilute any cell debris or other material left over from the DNA prep (or PCR), leading to better sequencing results. Amplicon Express can prepare your DNA for you at a very reasonable price.

Q- How should I submit DNA for sequencing if I have 15 or fewer samples? 16 or more samples?

15 or fewer samples: Please submit them in 1.5-mL microcentrifuge tubes sealed with parafilm.

16 or more samples: You may submit them in 8 or 12 strip 0.2-ml tubes or 96-well plates.

All strip caps and plate covers must be securely fastened to the tubes to prevent evaporation and/or leakage from the tubes. DNA samples do not need to be sent on ice, and can be sent via regular mail or other carrier service, but should be securely packaged to prevent damage during shipping.

Q- How should I label my tubes? Please label tubes containing DNA samples with the **name of the sample and the concentration stated in nanograms per microliter**. Likewise, label tubes containing primers with the name of the primer and the concentration stated in picomoles per microliter or the micromolar conc. (e.g.: 10 µM is equivalent to 10 picomole / µl).

Q- What is the best way to determine the concentration of my DNA sample?

By agarose gel verification: Verify the concentration of your sample on an agarose gel along with a mass standard to get a fairly good idea of the concentration of your DNA.

By fluorimeter: Fluorimetry readings will give a more accurate concentration of your DNA sample. However, they will not discriminate between any contamination and true products such as non-specific PCR product, genomic DNA, some salts (which can elevate or depress fluorescence readings), etc. In some cases, the contaminants can add to or even mask the true concentration of your sample.

By spectrophotometer: This method is probably the least reliable of the three, as it measures concentration by comparing the OD 260 / OD 280 ratio of your sample, and does not directly measure the DNA concentration or give you an idea of what may be contained in the sample. Any other contaminants in a DNA preparation that show fluorescence at this wavelength can change the result, giving an incorrect quantification.

We can quantitate your DNA template in our lab for \$2.50 per sample, which will allow us to use our internal quality control procedures to get you the best possible data.

Q- How should I prepare and purify my BAC and plasmid DNA for sequencing?

Plasmid DNA needs to be prepared with an alkaline lysis prep **and** an ion-exchange resin (such as the columns found in Qiagen's 'REAL' prep or Promega's 'Wizard' prep kits). (Generally, using only an alkaline lysis or boiling method of plasmid prep yields results too impure to sequence.)



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BAC DNA needs to be purified by an ultrapure maxi- or midi- prep (e.g. DNA prep kits made by Qiagen, Princeton Separations, Gibco BRL or Autogen). Please call us if you have questions concerning your BAC DNA preps. We have reliable technologies for preparing sequencing-grade BAC DNA for your project – call for a quote.

Q- Does Amplicon Express make direct sequencing on large clones like P1, PAC, BAC, COSMID, FOSMID or PHAGE? Yes. Let us know the size of your insert and we will help you find the most cost-effective way to proceed.

Q-How many bases can we expect for large insert clone sequencing? Generally, if the template is very clean, we can read 500 (+/-50) basepairs for one single-stranded sequencing run on an average template. "Non-average" templates include high G/C, high A/T, microsatellite and those with a long mononucleotide stretch.

Q- What primers does Amplicon Express supply? We provide T7, T3, M13F, M13R, S.TAG T7 Terminator, T7 Promoter and any others if they are the "universal primer" on a plasmid. We do not use SP6 or pBAD forward primers. For other vector primers please provide a map of the plasmid containing the primer and we will make it for free.

Q- How should I submit my custom primers? Please submit your custom primer at a concentration of 10 μ M (10 pm/ μ l). We need 2 μ L per sample, per reaction with a minimum volume of 25 μ L. All primers should be suspended either in 1X Tris or in distilled water.

Q- What about my PCR products? Can you sequence any PCR product? Almost. For us to sequence a PCR product, it must be a single band on a gel (to avoid mispriming by the sequencing primer and causing an unreadable 'double' sequence) at the concentrations stated above. Although we have had success using lower concentrations, we cannot guarantee the success of these reactions. Products showing more than one band on a gel can be gel purified to obtain a single product. However, it is **very important** to verify the concentration of the PCR product on an agarose gel **after** the purification procedure, as recovery of gel extracted DNA is almost never 100%. Additionally, the PCR product should not have any kind of fluorescent label (labeled primers or dNTPs) on it, as this would directly interfere with the sequencing reaction. Finally, the PCR product needs to be purified before the sequencing reaction in order to remove the excess primer and dNTPs left from the PCR. We prefer to perform this purification procedure (Exo/SAP enzymatic purification) in our lab, as it allows us to use our quality control procedures to insure that the data you receive is the very best. The price per reaction for Exo / SAP purification procedure can be found in our current price list at www.genomex.com. Another option is to use a size exclusion column.

Q- How many basepairs can I expect from one sequencing reaction of a PCR product? This depends on the concentration and quality of the initial PCR. In most instances, we can sequence 700 bp very cleanly. We have also had some templates where we have read over 800 bp very accurately.

Q- Are there some templates that are harder than others to sequence well? If so, what are they, and why? Although the sequencing chemistry we use is very robust, and has alleviated most of the difficulties inherent in using earlier sequencing chemistries, some templates are reticent to providing good sequence data:



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High G/C templates (>70% GC)—These templates may not stay denatured during the cycle sequencing reaction, making it difficult for the polymerase to incorporate dNTPs and dddNTPs into the growing sequence ladder. Compression can also be a problem in High G/C templates. Compression is caused by two DNA fragments of different sizes migrate to the same position in the gel. All fragments after the first bases showing compression generally do so as well, causing a sequence that may start out robustly to degrade rapidly.

High AT templates (>70% AT)—Secondary structures can form in these templates, causing poor polymerase binding to the template, leading to poor sequencing reads.

Samples with high salt concentrations

Different salts will inhibit the sequencing reaction at different concentrations. For instance, empirical evidence shows that sequencing reactions will be inhibited at EDTA concentrations of greater than 1 mM, and the length of read in a sequencing reaction will be noticeably reduced at NaCl concentrations of greater than 40 mM.

Repeatedly frozen and thawed or old templates DNA template may become degraded, leading to much shorter sequencing reads and poor quality data.

Sequences with long stretches of a single nucleotide

Usually, these are polyA sequences, and can be difficult for BigDye Terminator chemistry to overcome. However, we have techniques that will often allow us to overcome these mononucleotide stretches and obtain the sequence following it.

Please remember that if any difficulties arise, we will do our very best to get you the data that you need. For the best sequencing results, please handle your samples according to our recommendations, and call us if you have any questions regarding our services. Other anomalies do exist and we will try to keep you informed if any may appear in your samples.

Q- What will I be charged if a reaction fails? This does not happen very often and when it does, we make every effort to determine what went wrong. If our positive controls indicate an operator or machine error, we will re-sequence the sample at no charge. If it appears the sample is the problem, we will charge \$6 to cover our set-up expenses. We will then work with you to assure that subsequent reactions proceed more successfully.

Q- How can I obtain Amplicon Express' Sequencing order form? The order form is available on our website www.genomex.com or call and we will e-mail or fax it to you.

Q- Do I have to fill out the whole sample sheet? No, but we do need sample name, vector, insert size, conc./vol., primer name, and method of purification, FOR EACH REACTION! If you have more than a few samples we **highly** recommend you use our excel version of sequencing order forms. Many of our clients prefer this format to easily input information.

Q- Help I cannot see my chromatogram traces!! Where can I obtain the program to view my chromatogram traces?

To visualize and print the chromatogram traces, please point your favorite web browser to the following URLs to obtain free software for **Windows based computers**:

<http://www.basic.nwu.edu/biotoools/Chromas.html>



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Here is a website for a different program (TraceViewer).

<http://www.codoncode.com/TraceViewer/install.htm> (for academic users only)

And yet another program for Windows 95/98/NT:

<http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html>

Alternately you may download the ABIview program free from our website under the "DOWNLOAD" section.

If you are a **Mac user**, you may download the EditView program free from our website under the "DOWNLOAD" section.

Q- What if I need to read a .pdf file?

Please visit (for both MAC & Windows)

<http://WWW.adobe.com/prodindex/acrobat/readstep.html>

Q- Where can I get another copy of this document and more info about Amplicon Express? For all this and more, please visit our web page. www.genomex.com