

Troubleshooting Notes for DNA Sequencing

A few points to take in to consideration when you don't get optimal sequencing results.

DNA

Plasmids -

How old is your DNA? It will degrade over time with long term storage and many freeze/thaw cycles.

How clean is your DNA? Did you use a commercial kit to clean it up, could there be RNA contamination, EtoH contamination, salt from a storage buffer (full TE buffer) or an organic (phenol, chloroform) from a previous extraction? All these contaminants could seriously affect a sequencing reaction.

How did you quantitate your sample? Could the concentration be a little off, if you ran them out on an agarose gel, could your estimation be too high or too low?

Did you check to see if your insert is in the plasmid? Check the size on an agarose gel.

PCR products -

Are you sequencing directly from your PCR reaction? Secondary products and residual primer will directly affect your results.

How did you clean up your PCR product? A commercial kit or your own reagents? How old was the kit. How long have your reagents been around and again what is the buffer you have the product stored in?

Did you check the concentration after the clean up? Some kits do not have great recovery rates, often depending on the size of the PCR product.

How big is the PCR product? A 100bp product requires a vastly different amount of DNA in a reaction than a 1000bp product.

PRIMERS

Did you design your own primers? Have you tried them with other samples before trying to sequence with them.

Were they synthesized correctly, ie. 5' to 3', correct sequence used?

Were they old stocks? Again freeze/thaw cycles will degrade primers.

Are they stored in water or Buffer? ddH₂O is the wrong pH to store oligonucleotides and will give no buffering protection during storage at -20.

Have you checked the priming site? Did you check we are using the correct vector primers, there are so many out there!!!

Have you tried a PCR to ensure the priming site is not corrupted before sequencing?

Is there another primer we can try that has worked in the past?

When sequencing a PCR product Perkin-Elmer will tell you not to use your PCR primers as sometimes they just don't work. They will tell you to try an internal primer, this of course is expensive. We have mostly had good luck using the PCR primers, but be aware that there is no certainty they will work!