

Frequently Asked Questions (FAQ)

1 How much DNA do I need per reaction?

Accurate quantitation is critical!

Too much or too little template will result in poor sequence.

In our experience you should use:

dsPlasmid (3-6 kB) : 0.25 ug.

or dsPlasmid (6-10 kB) : 0.25-0.50 ug.

or dsPlasmid (10-20kb): 0.50-1.0 ug.

or dsPlasmid (>20 kB) – Contact us first.

PCR fragment: 5-10 ng per 100 bp (ex. 400 bp = 20-40 ng).

ssDNA: Use 50% of the appropriate dsDNA amount.

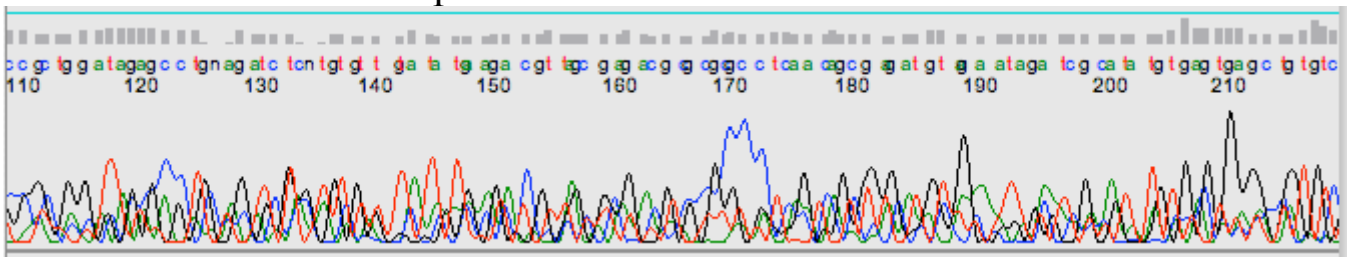
2 How close to the primer can I read?

Readable sequence usually begins 20-40 bases after the primer.

Sequence generated using Big Dye™ chemistry often starts closer to the primer.

3 What is a blank sample?

On the chromatogram view you will see data with undefined peaks close to the baseline. See example below:



Further the signal strength will be low. Signal strengths below 200 are considered blank. You can view your signal strength on finch by clicking on your data link. Once you do so the chromatogram details page will come up. Then notice the signal str. See below

Format	ABIF
Plate Label	n/a
Sample ID	n/a
Instr. Name	3730-2-15105-004
Run Stop	2006-04-25 18:34:26
# Lanes	48
Signal Strs	A=20,C=21,G=16,T=19
Matrix	n/a

4 Why was my sample blank?

This is our most common question and the most difficult to answer since a truly blank sample provides no information about what went wrong.

Listed below are the most common reasons for blank samples:

- Incorrect primer used or no primer annealing site in the template.
- Not enough template.
- Human Error
- Impurities in the template prep or primer.
- Extremely high GC content or template secondary structure.

See our troubleshooting instructions (downloadable) for ideas on how to correct blank samples.

5 When will my samples be ready?

Usually two business days unless there is a backlog.

6 How far will I be able to read my sequence?

The overall sequence quality is, of course, very dependent on DNA quality and signal strength. A typical run generates about 800 bases with an accuracy of 98-99% for the first 600 bases. As you read past 600 bases the accuracy decreases. High quality samples often generate accurate sequence to 700 bases and beyond.

7 Can I perform my own reactions and have you just run them on your instruments?

No. At this time the DNA Sequencing facility always performs both the sequencing reactions and runs them on our capillary instruments.

8 Are there volume discounts?

Yes. There are discounts for 48+, 96+ and 480+ samples submitted at one time. Contact us for specific instructions.

9 What is the trimmed length mean on my data?

(Example below)

```
ggcctccccennnaatctaatlncgctcttgttgtgtggaattgtgagcggataacaattt  
ccacaggaaacagctatgaccatgattacgccaagcttgcctgcagggtaatagcg  
aagaggcccgccaccgatcgcccttcccaacagttgcgagcctgaatggcgaatggcgcc  
tgatgcggtatcttctccttacgcatctgtgcggtatctcacaccgcatatggtgcactc  
tcagtacaatctactctaatcccacatagtttaagccagccccgacaccccccacacccc
```

The trimmed region on your text is what the software is calling low quality data.

10 What does the blue text mean?

This is referring to a vector region on a Finch database. You may disregard it.