Sequencing reaction preparation

Recommended Protocol:

Reagent	Recommended Volumes
BigDye (premix sequencing) DNA Sequencing Buffer 5x Primer (10 µM) Template DMSO (optional) Water	0.25 µl 2 µl 0.38 µl (3.2pmol) Table below* 0.1 µl** **Up to 12 µl (Final Volume)**

**Maximum 5% of the final reaction volum.
*Recommended DNA quantities per reaction by ABI:

Template	Quantity
PCR product	
100-200bp	1-3 ng
200-500bp	3-10 ng
500-1000bp	5-20 ng
1000-2000bp	10-40 ng
>2000bp	40-100 ng
Single Stranded	50-100 ng
Double Stranded	200-500 ng
Cosmid, BAC	0.5-1 ug
Bacterial Genomic DNA	2-3 ug

If you work with plasmids, you should calculate it:
ng DNA = 25ng x (size in Kb of your whole plasmid insert included)

Thermocycling conditions

The conditions for the Cycle Sequencing are:

94°C	3 min.	
96°C	10 sec.	
50°C *	5 sec.	**40 Cycles**
60°C	4 min.	
4°C	∞	

*** Ajust the annealing temperature according to the Tm of your primer.**

Submission

• Transfer the result of your sequencing reaction to a new 1.5 Eppendorf tube. **Be sure that your sample volume is at least 10 µl**. If you get volumes less than 10 µl please increase sequence reactions volume to 13 - 15 µl.

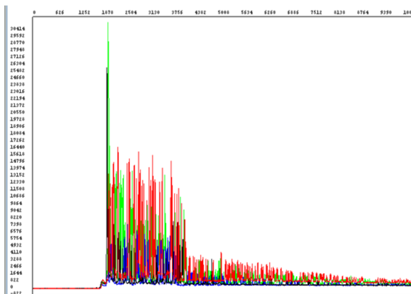
• Fill and send the online application and leave your tubes labeled with your user ID and sample name in the -20C fridge at the genomics Core Facility Lab. Please also leave a signed form in the laboratory.

Troubleshooting

Problem	Probable cause	Solution
Chromatogram starts well but stops abruptly (Figure 1)	Secondary structure (GC and AT rich templates can cause the DNA to loop and form hairpins) Linearized DNA (restriction enzymes may have cut an internal site)	Add (0.1ul) DMSO to the sequencing reaction to help relax the structure. Design primers close to the hairpin Run product out on an agarose gel to check
Chromatogram starts well but gradually dies (ski slope effect) (Figure 2)	Too much DNA template (overload of DNA leads to excessive number of short fragments)	Use less DNA template *
Noisy chromatogram (Figure 3)	Repetitive region (Especially GC and GT repeats, can cause the signal to fade either due to depletion or slippage or secondary structure)	Add (0.1ul) DMSO to the sequencing reaction. Sequence the complementary strand

Figure 1

Raw data



Electopherogram

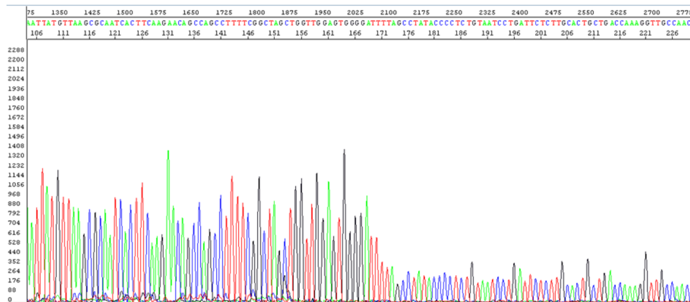
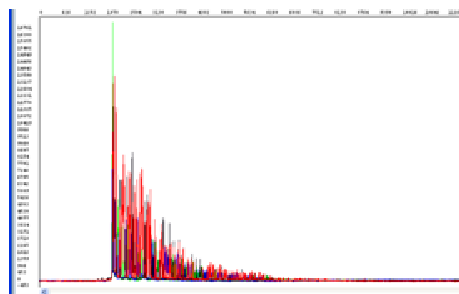


Figure 2



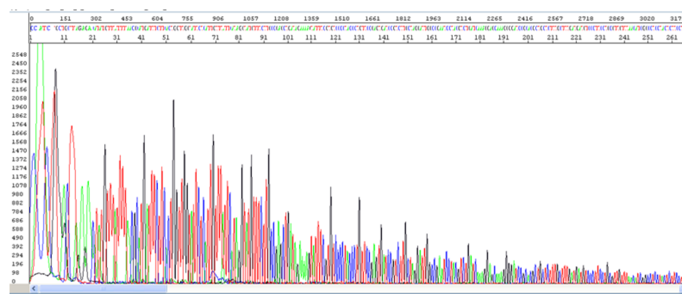
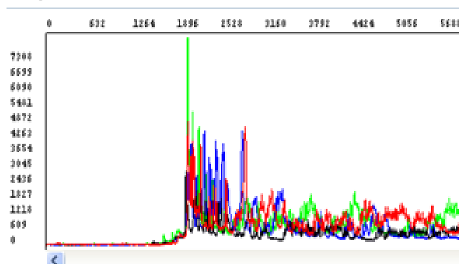
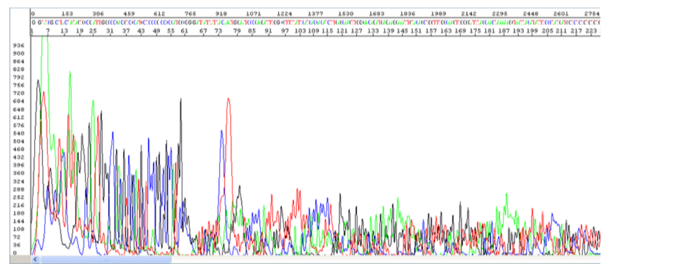


Figure 3





* Magnetic beads are used for sequencing clean up, which increases the efficiency of DNA purification. Excess of template DNA usually produces a decrease in the intensity after the first cycles. To prevent these problems we recommend users to change their sequencing setup and dilute the components concentration in order to find the best combination for your samples. As an example, the conditions shown below are used by some of our users.

1/32th:

0,12 µl BigDye
2,0 µl 5x BigDye buffer
0,8 µl primer (total 2,56 pmol per reaction)
1,4 µl template (given your concentration is about 100ng)
5,68 µl water
10,0 µl total reaction volume