Sequencing PCR products

1 Prepare working primer solutions

1. Get stock primers to 100pmol/ul in 1xTE (store this at -20°C).
2. To make your working solution (of 1.6 pmol/µl) add 3.2 µl of stock solution to each tube of 196.8ul of SDW.

2 Check DNA concentrations

1. Clean nanospec with 70% ETOH and add 1.2ul of SDW to the spec.
2. Open the nanospec program and allow it to initialize with the SDW.
3. Dab away the SDW and add 1.2µl of Elution Buffer. Click Blank.
4. Dab away the buffer and add 1.2µl of the sample to the spec and click Measure.

*Always use a new tip for each sample.

3 PCR Setup

1. Defrost your primers and Reddy mix on ice or in the fridge in advance.
2. Create a plate layout (as below).

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	etc								
B	2	10	18									
C	3	11	19									
D	4	12	20									
E	5	13	21									
F	6	14	22									
G	7	15	23									
H	8	16	24									

	1 X	n+1 X (e.g. n=24 samples below)
Primer f	2 µl (1.6 pmol/µl)	50
Primer r	2 µl (1.6 pmol/µl)	50
Reddy mix	5 µl	125
DNA sample	2 µl (1-5ng/µl)	
	11µl reaction	

3. Add 2µl of each DNA sample to its designated well in the PCR plate (add 1µl if >5ng/µl).
4. Prepare enough PCR solution to enable you to add 9µl of solution to each sample well. It is sensible to prepare a little extra to avoid running out of solution before you reach the final sample well (in the example above, if you have 24 samples then prepare solution for 25).
5. Add a drop of oil to each sample well. This stops the PCRs drying out.
6. Cover the plate with an adhesive sheet and run on the PCR machine on the required program. **e.g. Program Micro 60**
7. Make 1.5-2% agarose gel (e.g. 0.6g in 30ml TAE, 1µl ethidium bromide)
8. Remove 5µl of PCR product from each well and transfer to clean PCR plate wells ready for exosap cleanup.
9. Use the remaining oily PCR products for imaging on the gel. Layout left to right and flank with 100bp ladder. Keep gel pic for later reference. Hopefully, this will indicate successful PCRs.