DNA sequencing

(a) prepare terminator ready mix (this step probably isn't necessary ask someone for the mix)

20 ul BIG DYE
30 ul x5 sequencing buffer
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50 ul - keep at -20°C

(b) set up reaction in PCR tube:

1. mix:

8 ul terminator ready mix
3.2 pmol primer (eg 3.2 ul of 1 pmol/ml, 1:100 dilution of 100 uM standard stocks***)
200-500 ng of plasmid DNA (estimate from running 1 ul on a gel versus mw ladder)

add water to give a total of 20 ul

mix and spin down

2. run on sequen>seq program which is

35 cycles of 95°C for 30 sec
50°C for 20 sec
60°C for 4 min

3. transfer to larger 1.5 ml eppendorf tube and add

55 ul SDW
7.5 ul of 3M NaAc
150 ul of 100% etoh

leave at RT for 15'

4. spin 13k for 20 min (4°C) (** orient tubes so that you know where (invisible) pellet will be)

5. take off supernatant*. Wash with 1 ml 70% ethanol, respin (2 min 13 k) and take off supernantant* carefully. Re spin and take off remnant ethanol. [*keep supernatants in case you inadvertantly lose the pellet]

6. Dry by placing in 37°C heat block for 5-15 mins. Sample is now ready for the sequencing service.