

Helpful tips for DNA Sequencing

- When preparing a DNA template, always elute in H₂O, not TE buffer.
(*EDTA is a potential inhibitor in the reaction, and can cause poor or failed sequence data!*)
- Check the template concentration and quality using the Nanodrop spectrophotometer; the A₂₆₀/A₂₈₀ ratio should be 1.8-2.0, and the A₂₆₀/A₂₃₀ ratio should be 1.8-2.2.

- Standard reaction conditions:

Plasmid DNA template	300-500 ng
Primer	4 pmol/uL
BigDye reaction mix	8 uL*
H ₂ O	20 uL q.s.

*NOTE: 4uL of Big Dye plus 4uL of Seq. Dilution Buffer also work well

- Standard thermal cycling conditions:

96° C	30 seconds	1 cycle
96° C	10 seconds	
50° C	5 seconds	25 cycles
60° C	4 minutes	1 cycle
4° C	Hold	

- Remember that only 1 primer is used in the cycle sequencing reaction. Two primers will result in double signal, and the instrument can not decipher the peaks.

If a sample is **GC rich**, we recommend the following cycling conditions

- GC rich thermal cycling conditions:

96° C	1 minute	1 cycle
98° C	15 seconds	
55° C	1 second	30 cycles
62° C	4 minutes	1 cycle
60° C	2 minutes	1 cycle
4° C	Hold	

- Additionally, with GC rich or difficult templates (i.e. those with secondary structures or repeating regions), a product known as dGTP from Applied Biosystems** can be used in the reaction (**aliquotted and sold by FGC also):

Plasmid DNA template	300-500 ng <i>(see below if your template is PCR product)</i>
Primer	4 pmol/uL
dGTP	3 uL
BigDye reaction mix	3 uL
Sequencing dilution buffer	2 uL
H ₂ O	20 uL q.s.

- This reaction mixture would be run on the GC rich cycling protocol.
- If these do not work, you can try sequencing the PCR product (10-20ng PCR product for every 200bp of sequence should be used in the reaction)
- Do not try to directly sequence genomic DNA or other very large vectors. Perform a PCR reaction on the region of interest, purify the PCR product, and perform the cycle sequencing reaction using the purified PCR product as your DNA template. (10-20ng PCR product for every 200bp of sequence should be used in the reaction)
- If this still doesn't work, re-design the primer.
- *Always* clean up sequencing reactions using a magnetic bead cleanup kit or a spin column. (**Agencourt SPRI magnetic bead cleanup** or Princeton separations Centri-sep spin columns.) We strongly advise against performing an ethanol precipitation! The instrument is very sensitive to residual ethanol and salts, and a poor cleanup will produce what appears to be a failed reaction.