Protocol for sequencing directly from PCR products

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This protocol is appropriate for sequencing genes from isolates in culture and other *single template* DNA. Amplify the gene of interest and clean the pcr products with a pcr clean-up kit. Most researchers agree that the Qiagen MinElute Kit is the best. Quantify cleaned pcr products and make dilutions as needed for the appropriate concentration in the cycle sequencing reaction.

Recommended template concentrations

Size of PCR product	ABI recommended concentration (for 1X rxt.)
100-200 bp	1-3 ng
200-500 bp	3-10 ng
500-1000 bp	5-20 ng
1000-2000 bp	10-40 ng
>2000 bp	20-50 ng

I used 50 ng, 100 ng, and 200 ng of the same template in a 1/16X reaction for a 1500bp product. The 50 ng and 100 ng reactions both worked, but I got longer and cleaner sequence from the lowest concentrations.

Quantities for one 1/16X Cycle Sequencing reaction

BDT	0.5 μl
5X Reaction buffer	0.4 μl
Primer (15 μM, i.e. 15 pmoles/μl)	0.4 μl
DMSO	0.1 μl
Template (cleaned PCR product)	up to 50 ng
Molecular biology grade diH2O	as needed to bring total reaction volume to 6 μl

Thermocycle and clean cycle sequencing products by isopropanol precipitation as per standard JBPC protocol.