

DNA Template Quantity

Quantitating DNA DNA template quantitation is critical for successful sequencing reactions. The most common way to determine DNA quantity is to measure the absorbance (optical density or O.D.) of a sample at 260 nm in a spectrophotometer.

One O.D. unit is the amount of a substance dissolved in 1.0 mL that gives an absorbance reading of 1.00 in a spectrophotometer with a 1-cm path length. The wavelength is assumed to be 260 nm unless stated otherwise. A_{260} values can be converted into ng/ μ L using Beer's Law:

$$\text{Absorbance (260 nm)} = \text{sum of extinction coefficient contributions} \times \text{cuvette pathlength} \times \text{concentration}$$

The following formulas are derived from Beer's Law (Ausubel *et al.*, 1998):

- ◆ One A_{260} unit of single-stranded DNA contains 33 ng/ μ L.
- ◆ One A_{260} unit of double-stranded DNA contains 50 ng/ μ L.

Note Absorbance measurements of highly concentrated (O.D.>1.0) or very dilute (O.D.<0.05) DNA samples can be inaccurate.

DNA can also be quantitated by fluorometric analysis employing either Hoechst dye #33258 (Hofer, Inc., 1993) or Picogreen (Molecular Probes, Inc., 1995).

Amount to Use in Sequencing Reactions The amount of DNA template used in a sequencing reaction can affect the quality of the data. Too much template makes data appear top heavy with strong peaks at the beginning of the run that fade rapidly. Too little template or primer reduces the signal strength and peak height. In the worst case, the noise level increases so that bases cannot be called. Table 3-1 shows the recommended quantities for each sequencing chemistry.

Table 3-1 Recommended Ranges of DNA Template Quantity for Each Chemistry

Template	Cycle Sequencing Chemistry				
	Rhodamine Dye Terminator	dRhodamine Terminator	BigDye Terminator	Fluorescein/Rhodamine Dye Primer	BigDye Primer
PCR product:					
100–200 bp	1–3 ng	1–3 ng	1–3 ng	2–5 ng	2–5 ng
200–500 bp	3–10 ng	3–10 ng	3–10 ng	5–10 ng	5–10 ng
500–1000 bp	5–20 ng	5–20 ng	5–20 ng	10–20 ng	10–20 ng
1000–2000 bp	10–40 ng	10–40 ng	10–40 ng	20–50 ng	20–50 ng
>2000 bp	40–100 ng	40–100 ng	40–100 ng	50–150 ng	50–150 ng
single-stranded	100–250 ng	50–100 ng	50–100 ng	150–300 ng	150–400 ng
double-stranded	200–500 ng	200–500 ng	200–500 ng	300–600 ng	200–800 ng
cosmid, BAC	0.5–2.0 μ g	not recommended	0.5–1.0 μ g	0.5–2.0 μ g	0.5–1.0 μ g
bacterial genomic DNA	not recommended		2–3 μ g	not recommended	