Spectrophotometric Conversions:

Double-stranded DNA (ds DNA):
$A^{260} = OD^{260} = 1$ for a 50 µg/mL solution

Single-stranded DNA (ss DNA):
$A^{260} = OD^{260} = 1$ for a 33 µg/mL solution

RNA: $A^{260} = OD^{260} = 1$ for a 40 µg/mL solution

Reference: Freifelder, D., *Physical Biochemistry: Applications to Biochemistry & Molecular Biology*, W.H. Freeman and Company, CA, 1982, p. 494-536.

Useful Equations and Nucleic Acid Molecular Weight Data:

Absorbance = Molar Extinction Coefficient x Concentration x Path length

500 bp of double-stranded DNA = 325,000 Daltons
500 nt* of single-stranded DNA = 162,500 Daltons
Average MW of dNMP is 325 Daltons
(*nt = nucleotide)

Oligomer Quantitation:

For a 20-mer, a stock solution with $A^{260} = 1$ contains 5 nmol
5 nmol = 33 µg/(20 x 325)

For a 40-mer, a stock solution with $A^{260} = 1$ contains 2.5 nmol
2.5 nmol = 33 µg/(40 x 325)

Conversion of pmoles of primer to µg of primer:

Multiply pmoles by (length x 325)/1,000,000

Example: 10 pmoles of a 25-mer
(10 x 25 x 325)/1,000,000 = 0.081 µg primer

Conversion of µg of primer to pmoles of primer:

Multiply by 1,000,000/(length x 325)

Example:
0.1 µg of a 20-mer
(0.1 x 1,000,000)/(20 x 325) = 15.4 pmoles primer

Calculating Primer Concentrations for PCR Amplification:

Micromolar concentration of primer = pmoles/µL

Example 1:
20 pmoles of primer in 100 µL PCR mixture =
0.20 micromolar

Example 2:
Primer is 24 nucleotides in length and is dissolved in 0.1 mL of water

A 10 µL aliquot is diluted to 1.0 mL for A^{260} measurement:
$A^{260} = OD^{260} = 0.76$

The stock solution has an absorbance at 260 nm (A^{260}) of 76

The stock solution (0.1 mL) contains 7.6 A^{260} units

The base composition of the primer is A=6, C=6, G=6, T=6

The Molar Extinction Coefficient at 260 nm for the primer = a(16,000) + b(12,000) + c(7,000) + d(9,600)
where: a is the number of A's, b is the number of G's, c is the number of C's, d is the number of T's

The Molar Extinction Coefficient of the PCR primer is:
6(16,000) + 6(12,000) + 6(7,000) + 6(9,600) = 267,600

The Molar Concentration of the PCR primer stock solution is:
76/267,600 = 284 micromolar