Primer Quantitation

The following formula, which is derived from Beer's Law, converts A_{260} readings into pmol/µL concentrations:

$$C \text{ (pmol/µL or µM)} = (A_{260} \times 100)/(1.54n_A + 0.75n_C + 1.17n_G + 0.92n_T)$$

where:

C = concentration

n_X = number of residues of base x in the oligonucleotide

Oligonucleotide Molecular Weights

Molecular weight of a DNA oligonucleotide (sodium salt, pH≥7):

$$MW = (N_A \times 335.2) + (N_C \times 311.2) + (N_G \times 351.2) + (N_T \times 326.2) + P$$

where:

N_X = number of residues of base x in the oligonucleotide

P = –101.0 for dephosphorylated oligonucleotides, 40.0 for phosphorylated oligonucleotides

Primer Problems and Possible Causes

Table 3-2 Primer Problems and Possible Causes

Problems	Possible Causes
Poor priming resulting in weak or no signal	Melting temperature is too low due to low GC content and/or short primer length
	Secondary structure of the primer, particularly at the 3´ end
	Secondary structure of the template in the region of hybridization
	Incorrect primer concentration
	Priming site not present
Adequate signal strength with noisy data	Secondary hybridization site, which results in many extra peaks
	Impure primer. You may see a shadow sequence of N–1.

Custom Oligonucleotides

You can obtain custom primers from the MMI Core Facility Synthesis Service:

- Lab_phone: (503) 494-2472
- E-mail: core@ohsu.edu
- Online: www.ohsu.edu/core