PCR and Primer Design

Oligonucleotides are frequently used as primers in PCR. Unlike most large DNA, where the overall representation of the 4 bases averages out to about equal numbers of each, the base composition of an oligonucleotide (usually 15–30 bases) can vary greatly and affect the properties of the oligonucleotide. Key properties that are sequence dependent in oligonucleotides include extinction coefficient (1), A$_{260}$/280 ratio (1), electrophoretic migration (2), and ethidium bromide staining (2).

Use the free online OligoPerfect™ Designer software for primer design. Visit www.invitrogen.com and select OligoPerfect™ Designer from the Custom Primers menu.

References:

T$_m$ Calculations

Wallace Method (for oligos <18mers): T$_m$ = 2 x (A + T) + 4 x (G + C)

%GC Method: T$_m$ = 81.5 + 16.6 (log10[Na+] + 0.41[%GC] - [625/N])
N = length of oligo

Nearest neighbor (1) T$_m$ = (ΔH - 3.4 kcal)/((A + ΔS) + (R ln(C/4))) - 273.15 + 16.6 log10[salt]
ΔH is the sum of nearest neighbor enthalpy changes
A is the initiation constant of -10.8 cal/K° mole for non-self complementary sequences, -12.4 cal/K° mole for complementary sequences
ΔS is the sum of nearest neighbor entropy changes
R is the gas constant 1.987 cal/K° mole
C is the concentration of oligonucleotide (generally fixed at 250 pM) (2)

References: