Calculating DNA concentration

This technical bulletin describes the methodology that ACGT Corporation employs to calculate the extinction coefficients and concentrations of our DNA oligonucleotides. DNA can be quantified by measuring its absorbance of ultraviolet light at 260 nm and employing the Beer-Lambert Law. The following equation is used to calculate the concentration from absorbance values:

$A_{260} = \varepsilon_{260}\, l\, c$

Where:

A_{260} is the absorbance value at 260 nm
ε_{260} is the extinction coefficient of the oligonucleotide at 260 nm, its units are L/(mol · cm)
l is the pathlength in cm
c is the concentration in mol/L

The extinction coefficient of a given oligonucleotide must be known in order to determine the concentration. This can be calculated using a formula employing the nearest-neighbour model[1,2]. This takes into account the effects of adjacent nucleotides on UV absorption. The formula sums the extinction coefficients of each pair of nucleotides and subtracts the sum of the individual nucleotides with the exception of the extinction coefficients of the terminal nucleotides.

To illustrate, the extinction coefficient of the sequence ACGT can be calculated using the following formula:

$\varepsilon ACGT = \varepsilon AC + \varepsilon CG + \varepsilon GT - \varepsilon C - \varepsilon G$

Note that the individual coefficients for the 5' terminal A and 3' terminal T are excluded from this formula. The values for each pair of nucleotides are presented in table 1. The nucleotides in the first column are considered to be the first nucleotide in the pair. Note that the order matters for some nucleotide pairs. For example, GT is not equivalent to TG. The values for the individual nucleotides can be found in table 2.

Table 1. Extinction coefficients for nucleotide dimers in L/(mol · cm)

	A	C	G	T
A	27400	21200	25000	22800
C	21200	14600	18000	15200
G	25200	17600	21600	20000
T	23400	16200	19000	16800

Table 2. Extinction coefficients and formula weights of individual nucleotides

Nucleotide	ε260 in L/(mol · cm)	FW in g/mol
A	15400	313.21
C	7400	289.18
G	11500	329.21
T	8700	304.20

Once you have determined the extinction coefficient the concentration can be calculated using the first formula written above.

$c = A_{260} / (l \cdot \varepsilon_{260})$

For example, if the absorbance value for a solution of the sequence ACGT was measured to be 0.500 then,

$c = 0.500 / (1 \cdot 40300)$
$= 12.4\ \mu M$

Calculating Molecular Weight

To calculate the molecular weight of an oligonucleotide we apply the following formula:

MW = sum of FW – 61.96

Table 2 lists the formula weights of individual nucleotides when they are incorporated into an oligonucleotide. These values are available from our phosphoramidite supplier Glen Research[3]. This equation takes into account the fact that a synthesized oligonucleotide does not contain a 5' monophosphate.

Differences in extinction coefficients

The extinction coefficient calculated for an oligonucleotide is dependent on the values of the individual extinction coefficients used, which can vary depending on their source. The error in calculating DNA concentrations from these values can be 2-3%[4]. ACGT Corporation uses the above tables and formulas for calculating extinction coefficients and molecular weights.

References

1. Cantor C.R., Warshaw M.M., and Shapiro H. 1970. Oligonucleotide interactions. III. Circular dichroism studies of the conformation of deoxyoligonucleotides, Biopolymers 9, 1059-1077.
2. Fasman, G.D. (Ed.) 1975. Handbook of Biochemistry and Molecular Biology, Volume 1: Nucleic Acids, pp 589, 3rd edition, CRC Press.
3. Glen Research. Sep 15, 2005. Physical Data. Retrieved Dec 3, 2012, from http://www.glenresearch.com/catalog/physicaldata.html.
4. Cavaluzzi M.J., and Borer P.N. 2004. Revised UV extinction coefficients for nucleoside-5'-monophosphates and unpaired DNA and RNA, Nucleic Acids Res. 32, e13.