QUANTITATING NUCLEIC ACIDS

DNA or RNA (based on absorbance at 260 nm)

1. prepare a dilution of DNA or RNA sample in distilled water (typically 1:100 or 5:500 µl)
2. using UV light source, read absorbance at 260nm, may also check absorbance at 280 and 230nm (260/280 ratio should be roughly 2/1; 260/230 ratio should also approach 2/1)
3. to calculate concentration of original solution:

   A_{260} **x** conversion factor **x** dilution factor = original DNA concentration (µg/ml) absorbance unit

   the conversion factor for double stranded DNA is 50µg/ml; for single stranded DNA and RNA the conversion factor is 40µg/ml

The molecular weight of an average deoxynucleotide monophosphate (base) is 326.95. Therefore, the average basepair is 753.9. To calculate ,µmoles of ends from this value, for example, calculate the following:

(2 **x** µg DNA) + [753.9 **x** (size of double stranded DNA in bp)]

thus 1µg of pUC DNA corresponds to 2 x 1µg + [753.9 µg/µmol bp x 2676 bp] or 2µg + 2017436.4 µg/µmol = 1 x 10-6µmol

Oligodeoxynucleotides

1. calculate extinction coefficient (e) for oligonucleotide:
   a. tabulate number of occurrences of each base, A, C, G and T
   b. multiply results by the following values:

number of A's x	15.4 ml/µmole
number of C's x	7.3 ml/µmole
number of G's x	11.7 ml/µmole
number of T's x	8.8 ml/µmole

   c. the sum of these results is the extinction coefficient, ∈
2. determine absorbance of oligonucleotide solution at 260 nm
3. calculate concentration with the following formula:

   concentration = A260 ÷ ∈ (note: µmole/ml = mM)

NB. oligonucleotides are frequent supplied as lyophilized powders with amounts listed in O.D. units; one O.D. unit in this nomenclature corresponds to the A260 this sample would have were it resuspended in 1ml (roughly); this information can be used with the above formulae to estimate what the concentration of oligo would be in such a solution and a more preferred concentration of oligo can be prepared based on this information;

for example:

an oligo has a ∈ of 262.9 ml/µmole; 3 O.D. units were supplied; therefore, a 1ml solution would have a concentration of 3 ÷ 262.9 ml/µmole = 11.4 µM to obtain a, say, 20µM solution calculate $C_1V_1 = C_2V_2$:

1ml **x** 11.4 µM = ? **x** 20 µM; (1ml **x** 11.4µM)/20µM = 0.570ml

resuspend in 570µl!