Using spectrophotometer to quantitate DNA and RNA
MFT, 12/30/02 – taken from Maniatis, E.6

If sample is pure (i.e. without significant amounts of contaminantes such as proteins, phenol, agarose, or other nucleic acids), can use spec to measure amount of UV irradiation absorbed by the bases.

For quantitating DNA or RNA, readings should be taken at wavelengths of 260 nm and 280 nm. The reading at 260 nm allows calculation of the concentration of nucleic acid in the sample.

1 O.D. at 260 nm for double-stranded DNA = 50 ng/ul of dsDNA
1 O.D. at 260 nm for single-stranded DNA = 20-33 ng/ul of ssDNA
1 O.D. at 260 nm for RNA molecules = 40 ng/ul of RNA

The reading at 280 nm gives the amount of protein in the sample.

Pure preparations of DNA and RNA have OD_{260}/OD_{280} values of 1.8 to 2.0, respectively. If there is contamination with protein or phenol, this ratio will be significantly less than the values given above, and accurate quantitation of the amount of nucleic acid will not be possible.

So typically, dilute sample 1 ul in 100 ul so the dilution factor is 100. Put whole 100 ul in spectrophotometer cuvette. The DNA concentration read will then be:

\[ \text{OD}_{260} \times 50 \text{ ng/ul} \times \text{dilution factor} \]

For example, if have \text{OD}_{260} = 1.6. Then the concentration is:

\[ 1.6 \times 50 \text{ ng/ul} \times 100 = 8000 \text{ ng/ul or 8 ug/ul} \]