DNA quantification using absorption spectroscopy
Reliable quantitation of nanogram and microgram amounts of DNA and RNA in solution is essential to researchers in molecular biology. Absorbance measurements are straightforward as long as any contribution from contaminants and buffer components are taken into account. Absorption of the sample is measured at several different wavelengths to assess purity and concentration of nucleic acids. $A_{260}$ measurements are quantitative for relatively pure nucleic acid preparations in microgram quantities. Absorbance cannot discriminate between DNA and RNA; however, the ratio of $A$ at 260 and 280 nm can be used as an indicator of nucleic acid purity. Proteins, for example have a peak absorption at 280 nm that will reduce the $A_{260}/A_{280}$ ratio. Absorbance at 325 nm indicates particulates in the solution or dirty cuvette; contaminants containing peptide bonds or aromatic moieties such as protein and phenol absorb at 230 nm. This protocol is designed for a single beam ultraviolet to visible range (UV-VIS) spectrophotometer.

**Materials:**
distilled water (or other appropriate buffer)
DNA sample to be quantitated
Quartz semi-micro spectrophotometer cuvettes (1-cm pathlength)
Single-beam spectrophotometer (ultraviolet to visible)

**Procedure:**
1. Turn on the spectrophotometer. Allow it to boot. Go to USER PROGRAM, select program #3 Fix λ. Allow UV lamp to warm up for 15 minutes.
2. Make dilution of DNA sample: 4 uls DNA sample + 76 uls dH$_2$O. (This is a dilution of your DNA sample as follows: 4:76 or 4/80 or 0.05) (*When measuring RNA use 3 uls/72 uls dH$_2$O*)
2. Pipet 100 uls of dH$_2$O into a quartz cuvette. Place the cuvette in the spectrophotometer. Press blank. Spec. will blank at 280nm, 260 nm, 325 nm, 230 nm. Remove blank cuvette and insert cuvette containing DNA sample or standard suspended in the same solution as the blank. Press read sample. Take readings and record results:

   280 nm: ________________

   260 nm: ________________ = [DNA] ug/ul or mg/ml

   325 nm: ________________

   230 nm: ________________