QUANTITATION OF NUCLEIC ACIDS

CAUTION: Please see Appendix 12 for appropriate handling of materials marked with <!>.

Two types of methods are widely used to measure the amount of nucleic acid in a preparation. If the sample is pure (i.e., without significant amounts of contaminants such as proteins, phenol, agarose, or other nucleic acids), spectrophotometric measurement of the amount of UV irradiation absorbed by the bases is simple and accurate. If the amount of DNA or RNA is very small or if the sample contains significant quantities of impurities, the amount of nucleic acid can be estimated from the intensity of fluorescence emitted by ethidium bromide or Hoechst 33258. A summary of the methods commonly used to measure the concentrations of DNA in solution are listed in Table A8-4. More detailed discussion of the methods follows after the table.

TABLE A8-4 Measuring Nucleic Acid Concentrations

METHOD	INSTRUMENT	COMMENTS
Absorbance at 260 nm	spectrophotometer	Useful only for highly purified preparations of nucleic acid, since it detects any compound that absorbs significantly at 260 nm, which includes, for example, DNA, RNA, EDTA, and phenol. The ratio of absorbance at 260 nm and 280 nm is often used as a test for contamination of a preparation of DNA and RNA with protein. Despite its popularity, this test is of questionable worth. Nucleic acids absorb so strongly at 260 nm that only a significant level of protein contamination will cause a significant change in the ratio of absorbance at the two wavelengths (Warburg and Christian 1942; Glasel 1995; Manchester 1995, 1996; Wilfinger et al. 1997) (please see the panel on **ABSORPTION SPECTROSCOPY OF NUCLEIC ACIDS** on the following page). The specific absorption coefficients of both DNA and RNA are affected by the ionic strength and the pH of the solution (Beaven et al. 1955; Wilfinger et al. 1997). Accurate measurements of concentration can be made only when the pH is carefully controlled and the ionic strength of the solution is low. It is difficult to measure the absorbance of small volumes of solution and the method is reliable only over a fairly narrow range of concentrations (5 µg/ml to 90 µg/ml).
Emission at 458 nm in the presence of Hoechst 33258	fluorometer	Hoechst 33258 is one of a class of *bis*-benzimidazole fluorescent dyes that bind nonintercalatively and with high specificity to double-stranded DNA. After binding, the fluorescent yield increases from 0.01 to 0.6 (Latt and Wohlleb 1975); Hoechst 33258 is therefore a useful fluorochrome for fluorometric detection and quantitation of double-stranded DNA. Hoechst 33258 interacts preferentially with A/T-rich regions of the DNA helix, with the $\log_{10}$ of the intensity of fluorescence increasing in proportion to the A+T content of the DNA (Daxhelet et al. 1989). The fluorescent yield of Hoechst 33258 is approximately threefold lower with single-stranded DNA (Hilwig and Gropp 1975). Fluorometry assays with Hoechst 33258 do not work at extremes of pH and are affected by both detergents and salts (Van Lancker and Gheyssens 1986). Assays are therefore usually carried out in 0.2 M NaCl, 10 mM EDTA at pH 7.4. The concentration of DNA in the unknown sample is estimated from a standard curve constructed using a set of reference DNAs (10–250 ng/ml) whose base composition is the same as the unknown sample. The intensity of emission is nearly linear over a 1000-fold range of DNA concentrations. The DNAs must be of high molecular weight since Hoechst 33258 does not bind efficiently to small fragments of DNA. All DNAs and solutions must be free of ethidium bromide, which quenches the fluorescence of Hoechst 33258. However, because Hoechst 33258 has little affinity for proteins or rRNA, measurements can be carried out using cell lysates or purified preparations of DNA (Cesarone et al. 1979, 1980; Labarca and Paigen 1980).
Dipstick (a kit from Invitrogen)		This method is good only for solutions containing low concentrations of DNA and RNA (<10 µg/ml), and is both expensive and relatively slow (30–40 minutes).
Ethidium bromide spot test	UV transilluminator	A fast and sensitive method that utilizes the UV-induced fluorescence emitted by intercalated ethidium bromide molecules. The DNA preparations under test are spotted onto an agarose plate containing 0.5 µg/ml ethidium bromide. A series of DNAs of known concentration are used as standards. Because the amount of fluorescence is proportional to the total mass of DNA, the quantity of DNA can be estimated by comparing the light emitted at 590 nm by the test preparations and the standards. The results of the assay can be recorded on film. In a similar, older test, developed in the early 1970s, DNA samples and standards are spotted onto a sheet of Saran Wrap, mixed with a dilute solution of ethidium bromide, and photographed. The chief problem with the method is that it is sensitive to interference by RNA.