Bio 181 Handout

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QUANTITATION OF DNA AND RNA

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 ultraviolet 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.

Spectrophotometric Determination of the Amount of DNA or RNA

For quantitating the amount of 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. An OD of 1 corresponds to approximately 50 μg/ml for double-stranded DNA, 40 μg/ml for single-stranded DNA and RNA, and ~20 μg/ml for single-stranded oligonucleotides. The ratio between the readings at 260 nm and 280 nm (OD_{260}/OD_{280}) provides an estimate of the purity of the nucleic acid. Pure preparations of DNA and RNA have OD_{260}/OD_{280} values of 1.8 and 2.0, respectively. If there is contamination with protein or phenol, the OD_{260}/OD_{280} will be significantly less than the values given above, and accurate quantitation of the amount of nucleic acid will not be possible.

Ethidium Bromide Fluorescent Quantitation of the Amount of Double-stranded DNA

Sometimes there is not sufficient DNA (<250 ng/ml) to assay spectrophotometrically, or the DNA may be heavily contaminated with other substances that absorb ultraviolet irradiation and therefore impede accurate analysis. A rapid way to estimate the amount of DNA in such samples is to utilize the ultraviolet-induced fluorescence emitted by ethidium bromide molecules intercalated into the DNA. Because the amount of fluorescence is proportional to the total mass of DNA, the quantity of DNA in the sample can be estimated by comparing the fluorescent yield of the sample with that of a series of standards. As little as 1–5 ng of DNA can be detected by this method.

Cautions: Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when working with solutions that contain this dye. After use, these solutions should be decontaminated by one of the methods described on pages E.8–E.9.

Ultraviolet radiation is dangerous, particularly to the eyes. To minimize exposure, make sure that the ultraviolet light source is adequately shielded and wear protective goggles or a full safety mask that efficiently blocks ultraviolet light.