QUANTITATION OF DNA AND RNA

Two methods are widely used to measure the amount of DNA or RNA in a preparation. If the sample is pure (i.e., without significant amounts of contaminants such as protein, 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.

Spectrophotometric Determination 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 oligonucleotides. The ratio between the readings at 260 nm and 280 nm (OD_{260}/OD_{280}) provides an estimate for the purity of the nucleic acid. Pure preparations of DNA and RNA have OD_{260}/OD_{280} 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 UV-absorbing substances that impede accurate analysis. A rapid way to estimate the amount of DNA in such samples is to utilize the UV-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.

Plastic Wrap Method

1. Stretch a sheet of plastic wrap over a UV transilluminator or over a black sheet of paper.

2. Spot 1–5 μl of your DNA sample onto the plastic wrap.

3. Spot equal volumes of a series of DNA concentration standards (0.5–20 μg/ml) in an ordered array on the plastic wrap.

4. To each spot add an equal volume of TE containing 2 μg/ml of ethidium bromide. Mix by pipetting.

5. Photograph the spots using short-wavelength, UV illumination (see page 162). Estimate the concentration of DNA by comparing the intensity of fluorescence in the sample with that of the standard solutions.

Agarose Plate Method

Contaminants that may be present in the DNA sample can either contribute to or quench the fluorescence. To avoid these problems, the DNA samples can be spotted onto the surface of a 1% agarose slab containing 0.5 μg/ml of ethidium bromide. Allow the gel to stand at room temperature for a few hours so that small contaminating molecules have the chance to diffuse away. Photograph the gel as described above.

Minigel Method

Electrophoresis through minigels (see page 163) provides a rapid and convenient way to measure the quantity of DNA and to analyze its physical state at the same time. This is the method of choice if there is a possibility that the samples may contain significant quantities of RNA.

1. Mix 2 μl of the DNA sample with 0.4 μl of gel-loading buffer IV (bromophenol blue only; see page 455) and load into a slot in an 0.8% agarose minigel containing 0.5 μg/ml of ethidium bromide.

2. Mix 2 μl of each of a series of standard DNA solutions (0.5–50 μg/ml) with 0.4 μl of gel-loading buffer. Load the sample into the gel.

   Note. The standard DNA solution should contain a single species of DNA, approximately the same size as the unknown DNA.

3. Carry out electrophoresis until the bromophenol blue has migrated approximately 1–2 cm.

4. Destain the gel by immersing it for 5 minutes in electrophoresis buffer containing 0.01 M $MgCl_2$.

5. Photograph the gel using short-wavelength, UV irradiation. Compare the intensity of the fluorescence of the unknown DNA with that of the DNA standards and estimate the quantity of DNA in the sample.