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Quantitation of Double-stranded DNA Using Ethidium Bromide

Sometimes there is not sufficient DNA (<250 ng/ml) to assay spectrophotometrically, or the DNA may be heavily contaminated with other substances that absorb UV irradiation and therefore impede accurate analysis. A rapid way to estimate the amount of DNA in such samples is to use 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. For more information on ethidium bromide, please see Appendix 9 and Chapter 5, Protocol 2.

Saran Wrap Method Using Ethidium Bromide or SYBR Gold

1. Stretch a sheet of Saran Wrap over a UV transilluminator or over a sheet of black paper.
2. Spot 1–5 µl of the DNA sample onto the Saran Wrap.
3. Spot equal volumes of a series of DNA concentration standards (0.1, 2.5, 5, 10, and 20 µg/ml) in an ordered array on the Saran Wrap.

   The standard DNA solutions should contain a single species of DNA approximately the same size as the expected size of the unknown DNA. The DNA standards are stable for many months when stored at –20°C.

4. Add to each spot an equal volume of TE (pH 7.6) containing 2 µg/ml ethidium bromide or an equal volume of a 1:5000 dilution of dimethylsulfoxide <!> (DMSO)/SYBR Gold <!> stock. Mix by pipetting up and down with a micropipette.
5. Photograph the spots using short-wavelength UV illumination for ethidium bromide, or 300-nm transillumination for SYBR Gold (please see Chapter 5, Protocol 2). Estimate the concentration of DNA by comparing the intensity of fluorescence in the sample with that of the standard solutions.

Minigel Method

Electrophoresis through minigels (please see Chapter 5) 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; please see Table A1-6 in Appendix 1) and load the solution into a slot in a 0.8% agarose minigel containing ethidium bromide (0.5 µg/ml).

   SYBR Gold is too expensive to use routinely in this technique.

2. Mix 2 µl of each of a series of standard DNA solutions (0, 2.5, 5, 10, 20, 30, 40, and 50 µg/ml) with 0.4 µl of Gel-loading buffer IV. Load the samples into the wells of the gel.

   The standard DNA solutions should contain a single species of DNA approximately the same size as the expected size of the unknown DNA. The DNA standards are stable for many months when stored at –20°C.

3. Carry out electrophoresis until the bromophenol blue has migrated ~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 (please see Chapter 5). Compare the intensity of fluorescence of the unknown DNA with that of the DNA standards and estimate the quantity of DNA in the sample.