Rapid determination of DNA concentration in multiple samples

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DNA fingerprinting with multilocus probes is a powerful method of determining paternity and can also be used for personal identification in forensic casework (1, 2). Robust protocols have been developed for the process and it has been found necessary to quantify the amount of DNA in a sample both to ensure complete digestions with Hinf I and to obtain consistent lane loading. Commonly used spectrophotometric methods of quantification are time consuming and are prone to error as a result of the difficulties in pipetting small volumes of viscous, high molecular weight DNA. The presence of RNA also interferes with the measurement. Labarca and Paigen (3) have described the use of a fluorescent dye, Hoechst 33258, which binds specifically to DNA and displays enhanced fluorescence on binding to DNA. Initial experiments using a single cell fluorimeter (Hoefer TJ100) showed that accurate quantification was possible but the method was too time consuming when processing multiple samples (>50). We have investigated the use of a fluorescent microtitre plate reader to simplify the procedure. DNA was isolated and restricted with Hinf I as described previously (1). The DNA concentration was determined by UV spectrophotometry and a series of dilutions was prepared. Aliquots (4 μ l) were placed in the wells of a black microtitre plate. Hoechst 33258 (100 μ l, 1 μ g/ μ l in 10mM Tris HCl, pH 7.5, 100mM NaCl, 1mM EDTA) was added to the wells, the tray was shaken manually for 10 seconds and then placed in a Titretek Multiskan II (Flow Laboratories).

Figure 1

Excitation is at 360nm and emission is measured at 405nm. The instrument was interfaced with an IBM PC XT and the DNA concentrations were calculated automatically using the Titretek software. A linear response was observed in the range 250ng - 2000ng (Figure 1). The signal is stable for up to 2 hours. Standards are analysed on every tray and over 40 samples can be analysed in duplicate. Although the method was developed to be used in the fingerprinting process, the method would be of use in any situation where a large number of samples have to be analysed.

References

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2. Jeffreys, A J; Wilson, V; Thein, S L (1985) Nature 314, 67-73.
3. Labarca, C and Paigen, K (1980) Analytical Biochemistry 102, 344-352.