

How do I determine the concentration, yield and purity of a DNA sample?

DNA yield can be assessed using various methods including absorbance (optical density), agarose gel electrophoresis, or use of fluorescent DNA-binding dyes. All three methods are convenient, but have varying requirements in terms of equipment needed, ease of use, and calculations to consider.

Absorbance Methods

The most common technique to determine DNA yield and purity is measurement of absorbance. Although it could be argued that fluorescence measurement is easier, absorbance measurement is simple, and requires commonly available laboratory equipment. All that is needed for the absorbance method is a spectrophotometer equipped with a UV lamp, UV-transparent cuvettes (depending on the instrument) and a solution of purified DNA. Absorbance readings are performed at 260nm (A_{260}) where DNA absorbs light most strongly, and the number generated allows one to estimate the concentration of the solution. To ensure the numbers are useful, the A_{260} reading should be within the instrument's linear range (generally 0.1–1.0).

DNA concentration is estimated by measuring the absorbance at 260nm, adjusting the A_{260} measurement for turbidity (measured by absorbance at 320nm), multiplying by the dilution factor, and using the relationship that an A_{260} of 1.0 = 50 μ g/ml pure dsDNA.

$$\text{Concentration } (\mu\text{g/ml}) = (A_{260} \text{ reading} - A_{320} \text{ reading}) \times \text{dilution factor} \times 50\mu\text{g/ml}$$

Total yield is obtained by multiplying the DNA concentration by the final total purified sample volume.

$$\text{DNA yield } (\mu\text{g}) = \text{DNA concentration} \times \text{total sample volume (ml)}$$

However, DNA is not the only molecule that can absorb UV light at 260nm. Since RNA also has a great absorbance at 260nm, and the aromatic amino acids present in protein absorb at 280nm, both contaminants, if present in the DNA solution, will contribute to the total measurement at 260nm. Additionally, the presence of guanidine will lead to higher 260nm absorbance. This means that if the A_{260} number is used for calculation of yield, the DNA quantity may be overestimated.

To evaluate DNA purity, measure absorbance from 230nm to 320nm to detect other possible contaminants. The most common purity calculation is the ratio of the absorbance at 260nm divided by the reading at 280nm. Good-quality DNA will have an A_{260}/A_{280} ratio of 1.7–2.0. A reading of 1.6 does not render the DNA unsuitable for any application, but lower ratios indicate more contaminants are present. The ratio can be calculated after correcting for turbidity (absorbance at 320nm).

$$\text{DNA purity } (A_{260}/A_{280}) = (A_{260} \text{ reading} - A_{320} \text{ reading}) \div (A_{280} \text{ reading} - A_{320} \text{ reading})$$

Strong absorbance around 230nm can indicate that organic compounds or chaotropic salts are present in the purified DNA. A ratio of 260nm to 230nm can help evaluate the level of salt carryover in the purified DNA. The lower the ratio, the greater the amount of thiocyanate salt is present, for example. As a guideline, the A₂₆₀/A₂₃₀ is best if greater than 1.5. A reading at 320nm will indicate if there is turbidity in the solution, another indication of possible contamination. Therefore, taking a spectrum of readings from 230nm to 320nm is most informative.

Fluorescence Methods

The widespread availability of fluorometers and fluorescent DNA-binding dyes makes fluorescence measurement another popular option for determining of DNA yield and concentration. Fluorescence methods are more sensitive than absorbance, particularly for low-concentration samples, and the use of DNA-binding dyes allows more specific measurement of DNA than spectrophotometric methods allows. Hoechst bisbenzimidazole dyes, PicoGreen® and QuantiFluor™ dsDNA dyes selectively bind double-stranded DNA. The availability of single-tube and microplate fluorometers gives flexibility for reading samples in PCR tubes, cuvettes or multiwell plates and makes fluorescence measurement a convenient modern alternative to the more traditional absorbance methods.

Fluorescence measurements are set at excitation and emission values that vary depending on the dye chosen. The concentration of unknown samples is calculated based on comparison to a standard curve generated from samples of known DNA concentration. Genomic, fragment and plasmid DNA will each require their own standard curves and these standard curves cannot be used interchangeably. Some fluorometers will generate standard curves and calculate the concentration of unknowns for you, eliminating the need for manual calculations. As with absorbance methods, dilution factor must be taken into account when calculating DNA concentration from fluorescence values.

Materials required for fluorescence methods are: a fluorescent DNA binding dye, a fluorometer to detect the dyes, and appropriate DNA standards. Depending on the dye selected, size qualifications may apply, and the limit of detection may vary. The usual caveats for handling fluorescent compounds also apply—photobleaching and quenching will affect the signal.

Agarose Gel Electrophoresis

Agarose gel electrophoresis is another way to quickly estimate DNA concentration. To use this method, a horizontal gel electrophoresis tank with an external power supply, analytical-grade agarose, an appropriate running buffer (e.g., 1X TAE) and an intercalating DNA dye along with appropriately sized DNA standards are required. A sample of the isolated DNA is loaded into a well of the agarose gel and then exposed to an electric field. The negatively charged DNA backbone migrates toward the anode. Since small DNA fragments migrate faster, the DNA is separated by size. The percentage of agarose in the gel will determine what size range of DNA will be resolved with the greatest clarity. Any RNA, nucleotides and protein in the sample migrate at different rates compared to the DNA so the band(s) containing the DNA will be distinct.

Concentration and yield can be determined after gel electrophoresis is completed by comparing the sample DNA intensity to that of a DNA quantitation standard. For example, if a 2µl sample of undiluted DNA loaded on the gel has the same approximate intensity as the 100ng standard, then the solution concentration is 50ng/µl (100ng divided by 2µl). Standards used for quantitation should be labeled as such and be the same size as the sample DNA being analyzed. In order to visualize the DNA in the agarose gel, staining with an intercalating dye such as ethidium bromide or SYBR® Green is required. Because ethidium bromide is a known mutagen, precautions need to be taken for its proper use and disposal.

Additional Resources

The information given here is excerpted from the DNA Purification Chapter of the Promega *Protocols and Applications* Guide, which contains reference information and protocols on basic molecular biology techniques. The guide is available on [our web site](#), from the [iTunes App Store](#) and on [Android Market](#).

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