DNA Quantification

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.

**Checking the quality by agarose gel electrophoresis**

Genomic DNA extraction reading at OD260 is equivalent to 50 µg/ml). A pure DNA solution has anOD260:OD280 ratio of 1.8 ± 1.The DNA concentration is calculated using the formula, DNA concentration (µg /µl) = OD at 260 nm × dilution times × standard value If OD260 is 1.00, it is equivalent to 50 µg DNA per ml (standard). We are taking 50 µl of DNA in 1 ml TE buffer, which means it is diluted 20 times. For example: if the spectrophotometer reading is 0.112 (say), then the concentration of DNA is calculated as, DNA concentration (µg/µl) = 0.112 × 20 × 50= 112 µg/ml most DNA extracts are not reasonably pure and therefore estimates of concentration using spectrophotometric measurements of UV absorption may be misleading because of the interference by RNA or non-nucleic acids contaminants. In this case, quantification of the DNA can be achieved by running the DNA samples on 1 % agarose gel stained with ethidium bromide (0.5 µg/ml)(Ethidium bromide is a fluorescent chemical that intercalates between base pairs in a double stranded DNA molecule). Aliquots of the DNA extracts are loaded [e.g. 7 µl of DNA mix (5 µl of each DNA sample and 2 µl of loading dye)] alongside a range [7 µl each of 5, 10, 25 and 50 ng/µl] of uncut lambda DNA standards. High molecular weight DNA will appear as a well-resolved band alongside the lambda DNA bands whilst the smearing below the band indicates mechanical or chemical degradation. A smeared band towards the bottom of the gel is an indication of the presence of RNA in the extract. A rough estimate of DNA content (±10 ng) may be obtained by comparing band intensities of the DNA extract and the standards by eye. Greater precision may be obtained with the aid of commercial gel imaging equipment and gel analysis software. However, most applications do not require that the amount of DNA be known exactly. Techniques such as RAPD analysis depend more upon ensuring a consistent amount of DNA from sample to sample.

**Procedure for spectrophotmetric measurement**

1. Switch on the spectrophotometer and allow warming up.
2. Turn on the mode to UV.
3. Set the wavelength to 280 nm.
4. Wash the cuvette with distilled H2O. Dry with tisuue.
5. using a micropipette, clean the inside of the cuvette by TE buffer.
6. Insert the cell containing 100 µl of TE into chamber.
7. Set the reading to zero.
8. Set wavelength to 260 and then set the reading to zero.
9. Remove the cuvette from its compartment and discard the TE.
10. Add 5.0 µI of the DNA sample in the cuvette.
11. Add 95 µl of TE. Mix solution thoroughly.
12. Insert the cuvette into the sample compartment and close the cover tightly.
13. Read the OD value directly from the screen. This gives the DNA concentration when multiplied by 1000. That is, if OD equals 0.200 then DNA concentration equals 0.200 x 50 x 20 = 200 µg/ml (50 because 1 unit OD corresponds to a concentration of 50 µg/ml and 20 because the DNA solution was diluted 20 X).

To assess purity of the samples:

1. Read the OD at 280 nm.
2. Compute for OD260/OD280. A ratio value of 1.8 suggests a highly pure preparation of DNA. Ratio values much less than that implies significant presence of contaminants (generally proteins) such that accurate quantitation of nulceic acids is not guaranteed.
3. Read the OD values for the other samples at 260 and 280 nm by repeating the steps described above. Make sure to wash the cuvette thoroughly with TE between DNA samples.

**Checking the quality by agarose gel electrophoresis**

DNA fragments produced through amplification or through restriction enzyme digestion are separated by using electrophoresis. Either agarose and polyacrylamide gels are used which act as a selective filter so that DNA molecules having different molecular sizes are separated into specific bands as they move away from the one electrode to other. DNA is negatively charged and will migrate to the positive electrode (anode) in an electric field. Because DNA molecules have a uniform charge:mass ratio, they exhibit similar electrophoretic migration properties in a resistance free medium. The migrated DNA is visualized under UV light with the help of an intercalating dye, ethidium bromide, which fluoresces when irradiated with UV. The size of the fragment generated can be estimated by comparing the electrophoretic mobility (distance migrated through the gel per unit time) of an unknown DNA molecule to the electrophoretic mobility of DNA molecules for which the sizes are known. The standard method used to separate, identify and purify DNA fragments is electrophoresis through agarose gels. The technique is simple, rapid to perform and capable of resolving mixtures of DNA fragments. The migration rate of DNA through agarose gels is additionally dependent upon the molecular size of the DNA, the agarose concentration, the conformation of the DNA and the applied current. A DNA fragment of a given size migrates at different rates through gels containing different concentrations of agarose. Thus, by using gels of different concentrations, it is possible to resolve a wide size-range of DNA fragment.