3. All reagents, glasswares and plasticware should be made RNAse free.

3.1.2.4. Quantification of DNA/ RNA

Nucleic acids can be quantified spectrophotometrically by reading the absorbance of the isolated DNA or RNA preparation at 260 nm. For this, dissolve a small amount of isolated DNA or RNA in TE buffer and read absorbance at 260 nm against TE buffer as blank. Quantify according to the formula:

1 O.D. corresponds to 50μg of double stranded DNA

1 O.D. corresponds to 40μg of single stranded RNA

The concentration of DNA and RNA can be calculated as follows:

i. For double stranded DNA:

Concentration of DNA = 50 x A_{260} x Dilution factor in sample solution (mg\ml)

ii. For RNA:

Concentration of RNA = 40 x A_{260} x Dilution factor in sample solution (mg\ml)

The amount of DNA can also be determined by the diphenylamine method as given under section 2.5.2 and RNA by Orcinol method as given under section 2.5.3.

3.1.2.5. Determination of DNA quality

The quality of DNA can be determined either by spectrophotometric determination or by visualizing the DNA by gel electrophoresis.

i. Spectrophotometric determination

Dissolve a small amount of isolated DNA in TE buffer and read absorbance at 260 and 280 nm against TE buffer as blank. The ratio of 260/280 should be 1.8-2.0 for a good quality pure DNA. Proteins are usually the major contaminants in nucleic acid extracts and these have absorption maxima at 280 nm. The ratio of absorbance at 260 and 280 nm, hence, provides a rough idea about the extent of contaminants in preparation.

However, if the 260/280 ratio is more than or less than 1.8 then it means that the DNA preparation is contaminated either with RNA, traces of proteins or with phenol.

ii. Gel electrophoretic determination

To confirm DNA isolation and quality gel electrophoresis can be performed as given under section 3.1.1.8. or as given below.

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Procedure

1. Prepare a 1% solution of agarose by melting 1 g of agarose in 100 mL of 0.5x TBE buffer in a microwave for approximately 2 min. Allow to cool for a couple of minutes then add 2.5 µl of ethidium bromide, stir to mix.
2. Cast a gel using a supplied tray and comb. Allow the gel to set for a minimum of 20 min at room temperature on a flat surface.
3. Load into separate wells 10 µl 1kb ladder and 5 µl sample + 5 µl water + 2 µl 6x Loading Buffer
4. Run the gel for 30 min at 100 V
5. Expose the gel to UV light and photograph (if necessary)

To confirm DNA quality, presence of a highly resolved high molecular weight band indicates good quality DNA, presence of a smeared band indicates DNA degredation.

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