

Principle of Quantification of DNA by Agarose Gel Electrophoresis

Agarose gel electrophoresis is a common method used to determine the quantity and approximate size of DNA molecules. The principle of quantification of DNA by agarose gel electrophoresis is based on the movement of DNA molecules through a gel matrix in response to an electric field. Here is a basic outline of the principle and steps involved:

Gel preparation: Agarose, a polysaccharide derived from seaweed, is dissolved in a buffer and heated to create a liquid gel solution. This solution is poured into a gel tray and a comb is inserted to create wells for sample loading. Once the gel solidifies, it forms a porous matrix that acts as a molecular sieve during electrophoresis.

Sample preparation: DNA samples to be quantified are mixed with a loading buffer containing a denser substance, such as glycerol or a tracking dye (e.g., bromophenol blue or xylene cyanol), which helps visualize the migration of DNA during electrophoresis. The samples are denatured by heating if necessary.

Loading and electrophoresis: The DNA samples, along with appropriate size markers or a DNA ladder, are loaded into the wells of the agarose gel. An electric field is applied across the gel by connecting the electrodes to the gel apparatus. The negatively charged DNA molecules migrate towards the positively charged electrode, moving through the gel matrix.

Separation and visualization: During electrophoresis, the DNA molecules separate based on their size. Smaller DNA fragments move faster and migrate farther through the gel than larger fragments. The progress of DNA migration is monitored visually or with the help of specialized detection methods, such as UV light and DNA-specific fluorescent dyes.

Size estimation and quantification: After electrophoresis, the gel is typically stained with a DNA-specific dye, such as ethidium bromide or SYBR Green, which binds to DNA and allows visualization of the DNA bands. The gel is photographed or scanned to capture the DNA band pattern. The size of the DNA fragments can be estimated by comparing their migration distance with that of the size markers. The intensity of DNA bands can provide a semi-quantitative estimation of the DNA quantity. For more accurate quantification, the DNA bands can be compared to a known concentration standard using specialized software or densitometry.

It is important to note that agarose gel electrophoresis provides a rough estimation of DNA quantity, primarily based on band intensity. For precise quantification, other methods such as spectrophotometry or fluorometry are typically employed. However, agarose gel electrophoresis remains valuable for assessing the size distribution and relative abundance of DNA fragments.