



DNA Quantification and Gel Electrophoresis: A Basic Overview

*Devanshi Limbani, Aastha Korat and Lovleen Kaur Bharaj

M. Sc. Scholar, Department of Biotechnology, College of Agriculture, JAU, Junagadh

*Corresponding Author's email: devanshilimbani56@gmail.com

DNA extraction is a fundamental step in molecular biology, but the success of downstream analysis depends on the quantity, purity and integrity of the recovered DNA. DNA quantification provides a numerical estimate of DNA concentration and an indication of sample purity, whereas agarose gel electrophoresis reveals DNA integrity and fragment-size distribution by separating molecules according to their electrophoretic mobility. A DNA ladder provides a reference for estimating fragment size, while band intensity and pattern can indicate the amount and structural condition of the sample. Together, these complementary techniques provide essential quality control before applications such as PCR, sequencing, genotyping and molecular marker analysis, linking DNA extraction with reliable molecular analysis.

Introduction

Every living organism carries a remarkable molecule called DNA (deoxyribonucleic acid). It contains the genetic information that helps determine how an organism develops, functions and reproduces. Although DNA is present in almost every cell, it is far too small to be seen with the naked eye. This creates a basic challenge in molecular biology: once DNA has been extracted from a biological sample, how can we know how much DNA we have and whether it is in good condition? Before DNA can be used for techniques such as PCR, DNA sequencing, genetic diversity analysis or molecular marker studies, researchers generally need to assess its quantity and quality. A DNA sample may contain a large amount of DNA but also contain unwanted substances, or the DNA may have been broken into smaller pieces. Therefore, simply obtaining DNA is only the first step; checking the DNA is equally important. Two simple laboratory approaches are particularly useful for this purpose. DNA quantification provides numerical information about the amount and purity of DNA, commonly through measurements of light absorption. Agarose gel electrophoresis, on the other hand, provides a visual way to examine DNA and separate DNA fragments according to their size. In this technique, DNA moves through a gel when an electric field is applied, producing visible bands after appropriate visualisation. Together, these methods allow scientists to move from an invisible DNA sample to measurable and visible evidence of its quantity, quality and size. Understanding these techniques therefore provides a useful foundation for understanding many modern applications of molecular biology.

Why Do We Need to Check DNA?

Extracting DNA from a cell is only the beginning of molecular analysis. Before DNA is used for further experiments, researchers need to know whether the extracted sample is present in sufficient quantity and whether it is of suitable quality. This is important because DNA samples can contain unwanted substances from the extraction process, and DNA molecules can also become fragmented or degraded. The quantity of DNA tells us how much DNA is present in a sample, usually expressed as a concentration such as nanograms per microlitre (ng/μL). The purity gives an indication of whether substances such as proteins or other compounds may be present along with the DNA (Shim *et al.*, 2024). However, these measurements alone cannot tell us whether the DNA molecules are physically intact. This is where agarose gel

electrophoresis becomes useful. By separating DNA through a gel and allowing the resulting bands to be visualised, researchers can obtain additional information about the DNA sample, including its integrity and, for DNA fragments, their approximate size. In simple terms, DNA quantification tells us “how much”, while gel electrophoresis helps us see “what it looks like.” Using both approaches gives a more complete picture of a DNA sample before it is taken forward for molecular analysis.

DNA Quantification: Measuring the Invisible

Once DNA has been extracted, one of the first questions a researcher asks is: How much DNA is actually present in the sample? This is known as DNA quantification. Measuring DNA concentration is important because many molecular biology techniques require DNA within a suitable concentration range. Knowing the concentration helps researchers determine how much sample should be used in subsequent experiments. One commonly used approach is UV-visible spectrophotometry. DNA absorbs ultraviolet (UV) light, particularly around 260 nm. A spectrophotometer measures how much light is absorbed by the sample, and this measurement can be used to estimate the amount of DNA present. The result is commonly expressed as nanograms per microlitre (ng/μL). The same measurement can also provide an indication of DNA purity. Absorbance at 280 nm is associated mainly with proteins. Therefore, comparing the absorbance at 260 and 280 nm gives the A260/A280 ratio, which is commonly used as a quick indicator of possible protein contamination in a DNA preparation. Around 1.8 indicates good quality DNA in a sample. However, this ratio should not be considered a complete measure of DNA quality; it is one part of the overall assessment. Modern microvolume spectrophotometers make this process particularly convenient because only a very small volume of DNA solution is required. Within a short measurement, researchers can obtain information about the sample's concentration and absorbance profile.

Agarose Gel Electrophoresis: Making DNA Visible

DNA is far too small to be seen directly, even after it has been extracted from a cell. Agarose gel electrophoresis provides a simple way to separate DNA molecules and make their distribution visible. It is one of the commonly used techniques in molecular biology for examining DNA samples. The technique uses a soft, jelly-like material called agarose. When agarose is heated in an appropriate buffer and then allowed to cool, it forms a porous gel. These tiny pores act as a molecular sieve through which DNA molecules can travel. DNA samples are loaded into small depressions called wells in the gel, and an electric field is then applied across it. DNA has an overall negative electrical charge because of its phosphate backbone. Therefore, when an electric field is applied (Semenov *et al.*, 2023), DNA molecules move through the agarose gel towards the positively charged electrode. As they travel through the microscopic pores, DNA molecules of different sizes move at different rates. Smaller DNA fragments generally pass through the pores more easily and travel farther, whereas larger fragments move more slowly and remain closer to the wells (Sowersby & Lewis, 2024).

How is the agarose gel prepared?

The concentration of agarose used depends on the size of DNA fragments that need to be separated. For many routine DNA analyses, a 1% agarose gel is a useful general-purpose choice. Lower concentrations, such as 0.7-0.8%, are generally more suitable for larger DNA fragments, while higher concentrations, such as 1.5-2%, provide better separation of smaller fragments.

The percentage refers to the weight of agarose in a given volume of buffer. For example, a 1% agarose gel contains:

1 g agarose + 100 mL electrophoresis buffer

For a smaller gel volume, the same proportion can be used:

0.5 g agarose + 50 mL buffer

The agarose is mixed with an appropriate electrophoresis buffer, such as TAE or TBE, and heated until the agarose dissolves completely and the solution becomes clear. After cooling

slightly, an appropriate DNA-binding stain may be incorporated according to the staining system being used. The molten agarose is then poured into a casting tray containing a comb, which creates the wells where DNA samples will later be loaded. Once the gel cools and solidifies, it is ready for electrophoresis.

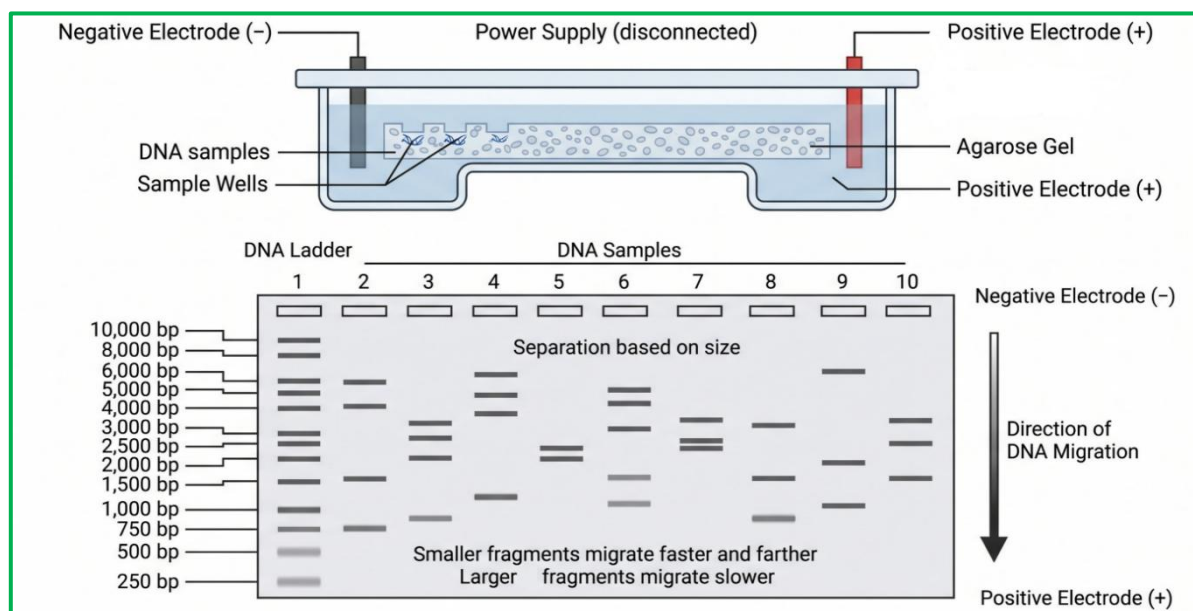


Figure 1: Agarose gel electrophoresis for DNA separation and visualisation

Figure 1 illustrates the separation of DNA fragments by agarose gel electrophoresis, where DNA molecules migrate through the agarose matrix under an applied electric field and become separated according to their size. Because DNA carries a negative charge, it moves towards the positive electrode. Smaller DNA fragments pass more easily through the pores of the agarose matrix and therefore migrate farther, while larger fragments move more slowly and remain closer to the wells. The resulting band pattern provides a visual representation of the DNA fragments present in the sample and can be compared with a DNA ladder to estimate their approximate sizes.

Role of Dyes in Gel Electrophoresis

DNA staining dyes bind to DNA and allow the separated DNA fragments to be visualised under suitable illumination. Commonly used dyes include ethidium bromide and several alternative fluorescent DNA stains (Tuo *et al.*, 2024). Table 1 shows main two types of dyes used.

Table 1: Dyes for DNA in gel electrophoresis

Dye	Purpose	When used
Tracking/ Loading dye	Helps the sample sink into the well and shows how far electrophoresis has progressed	Mixed with DNA before loading
DNA staining dye	Binds to DNA and makes the separated DNA bands visible	Added to gel/buffer or used after electrophoresis

Choosing the right gel concentration

The concentration of agarose is important because it affects how easily DNA molecules move through the gel. Table 2 shows different concentrations of gel for different purposes.

Table 2: Agarose concentration and DNA separation

Agarose concentration	Approximate DNA fragment size*	Relative pore size	DNA movement through gel	Best suited for
0.5%	~1-30 kb	Very large pores	Very fast	Very large DNA fragments
0.7%	~0.8-10 kb	Large pores	Fast	Large DNA fragments

Agarose concentration	Approximate DNA fragment size*	Relative pore size	DNA movement through gel	Best suited for
1.0%	~0.5-10 kb	Medium pores	Moderate	General-purpose DNA separation
1.2%	~0.4-7 kb	Medium-small pores	Moderate	Intermediate-sized fragments
1.5%	~0.2-3 kb	Small pores	Slower	Smaller DNA fragments
2.0%	~0.1-2 kb	Very small pores	Slow	Small DNA fragments
3.0%	~0.05-1 kb	Very small pores	Very slow	Very small DNA fragments

The DNA Ladder: A Molecular Ruler

After DNA has moved through the agarose gel, the separated molecules appear as bands. But simply seeing a band does not tell us how large the DNA fragment is. To estimate its size, scientists use a DNA ladder. A DNA ladder is a mixture of DNA fragments whose sizes are already known. These fragments are loaded into one lane of the gel alongside the unknown DNA samples. Because the known fragments move through the gel in a predictable way, they form a series of reference bands. Scientists can then compare the position of an unknown DNA band with the ladder to estimate its size, usually expressed in base pairs (bp) or kilobase pairs (kb). The idea is similar to using a ruler to measure an unknown length. Instead of centimetres or millimetres, the DNA ladder provides a scale based on the number of DNA base pairs. For example, if an unknown DNA band appears at approximately the same position as a 500 bp band in the DNA ladder, the fragment can be estimated to be around 500 base pairs long. This is an approximate measurement, rather than an exact determination.

How to Read a DNA Gel: Understanding the Bands

DNA bands become visible because the DNA-binding stain produces a fluorescent signal under suitable light. The stained gel is placed on a UV or blue-light transilluminator, depending on the stain used. The glowing bands can then be photographed and analysed using a gel documentation system.

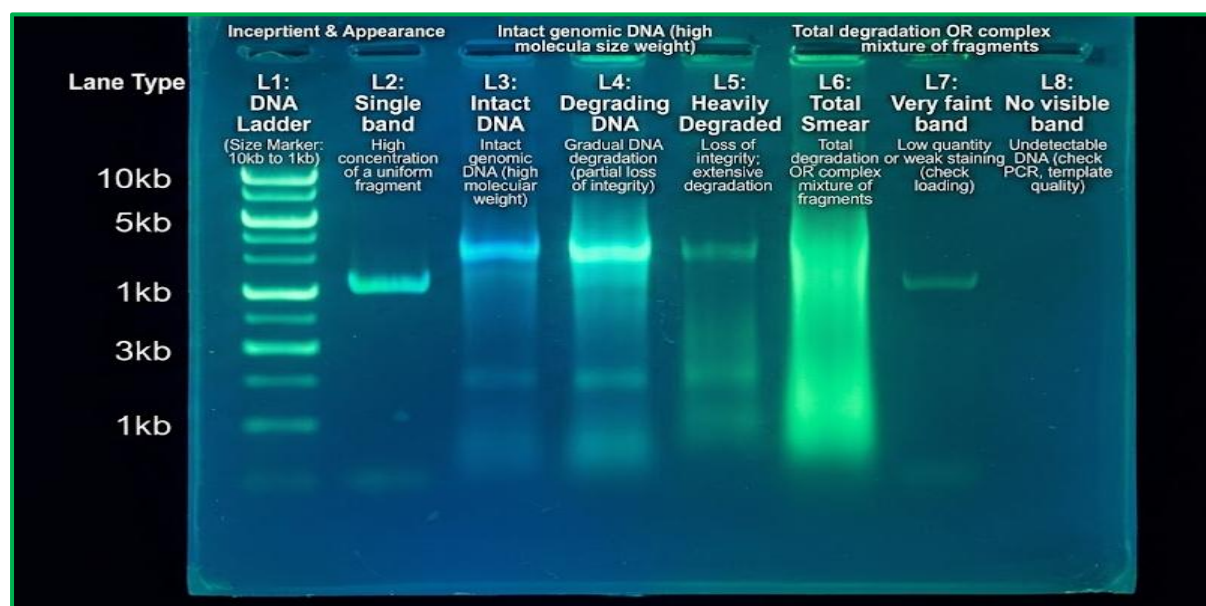


Figure 2: Comparison of fragment quality and loading outcomes. Lane 1: 1 kb DNA ladder; Lane 2: Pure PCR product (single, sharp band); Lane 3: High-molecular-weight intact genomic DNA; Lanes 4–5: Partially degraded genomic DNA showing downward trailing; Lane 6: Completely degraded DNA (total smear); Lane 7: Low-concentration DNA (faint band); Lane 8: Non-amplified/absent sample (no band).

Table 3 summarises common DNA patterns observed on an agarose gel, along with their possible interpretations. These patterns provide a visual indication of DNA quantity, integrity and fragment distribution, helping researchers assess the suitability of a DNA sample for subsequent molecular analyses.

Table 3: Common DNA patterns on an agarose gel (Sowersby & Lewis, 2024)

Appearance on gel	What it generally indicates	Possible interpretation
Single, sharp band	DNA concentrated in a particular size range	Relatively uniform DNA fragment
Several distinct bands	DNA fragments of different sizes	Multiple DNA products/fragments are present
Smear	DNA distributed across a range of sizes	May indicate DNA degradation or a mixture of fragments
Very faint band	Small amount of detectable DNA	Low DNA quantity or weak staining
Very bright/thick band	High amount of DNA	Sample may contain a relatively large amount of DNA
Band close to the well	Large DNA molecule/fragment	Large fragments migrate more slowly
Band farther from the well	Smaller DNA molecule/fragment	Smaller fragments migrate farther
No visible band	DNA not detected under the conditions used	DNA may be absent, too little, or not successfully visualised

From DNA Check to Molecular Research

Checking DNA is not the final goal - it is the quality-control step before further molecular research. Once researchers know that the DNA is present in sufficient quantity and is reasonably pure and intact, the sample can be used for a wide range of applications. For example, in PCR (polymerase chain reaction), DNA serves as the template for making millions of copies of a selected region. In DNA sequencing, good-quality DNA is needed to determine the order of nucleotides. In genotyping and molecular marker analysis, DNA is examined to identify genetic differences among individuals or varieties. The quality of DNA can directly affect these downstream experiments. DNA that is too dilute may not provide enough template, while degraded DNA or contaminants may interfere with amplification and other reactions. Therefore, checking DNA before an experiment helps researchers avoid unreliable results and unnecessary repetition.

Conclusion

DNA may be invisible, but its quantity, quality and integrity can be measured and visualised. Spectrophotometry tells us how much DNA we have, while agarose gel electrophoresis shows us what that DNA looks like. Together, these simple techniques turn an invisible molecule into measurable evidence, providing the starting point for PCR, sequencing, genotyping and many other molecular biology applications.

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