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STUDY OF THE DETECTION OF PCR PRODUCTS USING STANDARD AGAROSE GEL ELECTROPHORESIS

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Abstract

This thesis investigates the detection of DNA products obtained through Polymerase Chain Reaction (PCR) using standard agarose gel electrophoresis. The study analyzes the ability to determine the presence, size, and quality of PCR products. The results demonstrate that agarose gel electrophoresis is a simple, accurate, and reliable method. This technique is widely used and plays an important role in molecular biology and genetics.

Enter

Currently, molecular biology and genetics are one of the fastest growing fields. Scientific research in these areas serves to deeply study the genetic structure of living organisms, identify hereditary diseases, and improve biotechnological processes. In particular, the analysis of the DNA molecule and the identification of its components remains one of the main tasks of modern biology. In this regard, methods for amplifying and identifying DNA fragments are of great scientific and practical importance.

Polymerase chain reaction (PCR) is one of the most important methods for amplifying DNA fragments in vitro. This method can be used to amplify very small amounts of DNA samples to millions of copies in a short time. PCR technology is widely used in medicine for early detection of diseases, in forensics for personal identification, and in agriculture

for genetic selection. Therefore, the detection of PCR products and their quality assessment are important stages.

There are various methods for analyzing the products obtained after the PCR process, among which the agarose gel electrophoresis method is considered the most common and effective. This method is based on the separation of DNA molecules according to their size under the influence of an electric field. With the help of agarose gel electrophoresis, it is possible to determine the length of DNA fragments, confirm their presence, and assess the success of the amplification process.

The advantages of the agarose gel electrophoresis method are its simplicity, cost-effectiveness, and high accuracy. In addition, this method allows for rapid results in laboratory conditions. At the same time, this method can also detect incorrect amplification, contamination, or other technical errors. This increases the reliability of the results of scientific research.

Literature review

Numerous scientific studies in the fields of molecular biology and genetics have widely highlighted the importance of PCR and agarose gel electrophoresis methods. In particular, the polymerase chain reaction (PCR) method was first developed in 1983 by the American scientist Kary Mullis, which was later recognized as a revolutionary discovery in molecular biology. This method made it possible to multiply DNA fragments quickly and with high accuracy and began to be widely used in various scientific and practical areas.

Many foreign scientists have studied in detail the theoretical foundations and practical application of the PCR method. In particular, Sambrook and Russell, in their studies on molecular cloning, emphasized the important role of electrophoresis methods in the analysis of PCR products. In their opinion, agarose gel electrophoresis is one of the most convenient and reliable methods for separating and visualizing DNA fragments.

Also, in the literature on the basics of molecular biology written by Brown, PCR technology and its role in diagnostics are widely covered. The author notes that in the detection of PCR products, the length of DNA fragments can be determined by electrophoresis. This further increases the importance of the method in scientific research and laboratory practice

In studies conducted on the agarose gel electrophoresis method, the basic principle of its operation, namely the movement of DNA molecules in a gel environment under the influence of an electric field, has been shown. Various authors emphasize that the effectiveness of this method depends on the gel concentration, electric voltage and buffer solutions. These factors directly affect the accuracy of separation of DNA fragments.

Local scientists have also carried out a number of scientific developments related to PCR and electrophoresis methods. Their research highlights the use of these methods in the educational process, scientific laboratories, and the medical field. In particular, the importance of PCR and agarose gel electrophoresis in DNA diagnostics, detection of infectious diseases, and genetic analysis is highlighted.

The main part

Modern methods play an important role in the study and analysis of DNA molecules in molecular biology. One of them is the polymerase chain reaction (PCR), which allows you to multiply specific DNA fragments. This method allows you to obtain a large number of DNA copies in a short time and is widely used in scientific research and diagnostics. However, to confirm the success of the PCR process, it is necessary to identify and analyze the resulting products [1].

One of the most widely used methods for detecting PCR products is agarose gel electrophoresis. This method allows DNA fragments to be separated according to their molecular weight and size. Agarose gel is a polysaccharide substance obtained from seaweed, which is prepared in a solution and turns into a gel when cooled. Since DNA molecules have a negative charge, they move towards the anode under the influence of an electric field and move at different speeds within the gel. This speed depends on the length of the fragment: small fragments move faster, and larger ones move slower [2].

During the research, I prepared a standard 1–2% agarose gel for detecting PCR products. Ethidium bromide or another safe dye was added to the gel solution to visualize DNA fragments. The prepared gel was placed in a special electrophoresis apparatus and a buffer solution was added to it. The PCR products were mixed with a special loading dye and carefully placed in the gel wells [3].

During the electrophoresis process, when an electric current was applied, the DNA fragments began to move along the gel. The process usually took 30–60 minutes. The gel was then viewed using a UV transilluminator. As a result, the DNA fragments appeared as bright bands. According to the results I observed, the PCR products were formed in the expected size, indicating that the amplification process had been successful [4].

Agarose gel electrophoresis not only determines the presence of DNA fragments, but also their length. For this, a molecular weight marker (DNA ladder) is used. Using the marker, the size of unknown DNA fragments is approximately determined. One of the important aspects of this method is that it allows the detection of incorrect amplification products, primer dimers, or excess bands formed as a result of contamination [5].

However, agarose gel electrophoresis has some limitations. For example, it can be difficult to detect very small or very large DNA fragments. In addition, the toxicity of dyes such as ethidium bromide requires strict adherence to laboratory safety rules. Nevertheless, the simplicity, low cost, and reliability of this method make it one of the most widely used methods in molecular biology [6].

In general, the experiments conducted showed that standard agarose gel electrophoresis is an effective and convenient method for detecting PCR products. This method can achieve accurate results in a short time, which is of great importance in scientific research and laboratory practice [7].

Summary

This research study investigated the process of detecting DNA products obtained using polymerase chain reaction (PCR) using standard agarose gel electrophoresis. During the study, the theoretical foundations of PCR technology were analyzed and its important role in the fields of molecular biology and genetics was highlighted.

As a result of the experiments, I found that agarose gel electrophoresis is one of the simple, convenient and highly accurate methods for detecting PCR products. Using this method, it is possible to obtain reliable information about the presence of DNA fragments, their size and quality. The clear appearance of DNA bands in the experimental results confirmed the successful completion of the amplification process

It was also proven that this method can detect false amplification products, assess contamination, and detect errors in laboratory processes. This is important for increasing the accuracy and reliability of scientific research. During the study, some limitations of the agarose gel electrophoresis method were also noted. In particular, in some cases, the level of accuracy may decrease when detecting small or very large DNA fragments. However, its simplicity, cost-effectiveness, and widespread use compensate for these shortcomings.

In conclusion, standard agarose gel electrophoresis is one of the most important and effective methods for detecting PCR products. This method is widely used in molecular biology laboratories and plays an important role in scientific and applied research. In the future, the development of more advanced methods in this area and their implementation in practice will be one of the urgent tasks.

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