TRENDS IN AGRICULTURE SCIENCE
Popular Article
Vol.3 Issue 02 February 2024, Page: 1401-1405

Introduction to Plasmid Profiling in Molecular Biology

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https://doi.org/10.5281/zenodo.10625857

Abstract

The article provides an overview of plasmid profiling in molecular biology, emphasizing its significance in genetic diversity, antibiotic resistance, and microbial evolution. It covers the components of plasmids, types of plasmids, and techniques for plasmid DNA isolation and purification. Noteworthy methodologies such as electrophoretic separation and DNA concentration determination are also discussed.

Introduction

Plasmid profiling is a potent method in molecular biology that has transformed our knowledge of genetic diversity, antibiotic resistance, and microbial evolution. Small circular DNA molecules called plasmids, which are distinct from chromosomal DNA and are essential for the transfer of genetic material between bacteria. A method for analysing and characterizing plasmids is called "plasmid profiling." This technique has become important for figuring out the genetic diversity of microbes.

The Plasmid Profiling Technique

Plasmid profiling is a method for analysing and categorizing plasmids by size and characteristics. It begins with isolating plasmid DNA from bacterial cultures, followed by electrophoresis—a technique that uses an electric field to separate DNA fragments by size. The resulting banding patterns form distinct "fingerprints" for each plasmid, aiding in their identification and classification.

What is plasmid?

In the microscopic world of bacterial cells, plasmids, coined by scientist Joshua Lederberg, play a vital role in genetic diversity. These small, independent DNA molecules replicate autonomously, showcasing unique antibiotic-resistant genes. They contribute beneficial qualities with remarkable variability in gene content and size. Plasmids exhibit varying copy numbers, distributed during cell division, allowing a single cell to host multiple types simultaneously. Compatibility is key, as plasmids either coexist harmoniously or engage in

Published: 06 Feb. 2024
ISSN: 2583-7850

genetic rivalry based on differences or similarities in replicatory systems. The presence of plasmids shapes the genetic landscape of bacteria, influencing their survival and evolution.

Important components of plasmids

Plasmids are essential to molecular biology because of their complex architecture. The Origin of Replication (Ori) that facilitates the self-replication ensures plasmid survival and the quick dissemination of advantageous features. Resistance is provided by the antibiotic resistance gene, which influences agricultural and medical applications. Gene of interest is the fundamental unit of heredity, also constitute essential components of plasmids. Researchers strategically insert specific DNA sequences into plasmids to encode proteins, enabling the study of particular biological functions. Coupled with this is the Promoter, another critical DNA sequence that acts as a molecular switch, allowing the host cell to produce the proteins encoded by the inserted genes. DNA sequences that serve as molecular scissors called restriction sites, facilitating the precise customizations of plasmids. When combined, these elements provide a useful tool for expanding our knowledge of molecular biology and genetics.

Types of plasmids

Plasmids, diverse in types and functions, play crucial roles in bacterial biology. Fertility plasmids (F plasmids) facilitate genetic transfer through the sex pilus, vital for bacterial reproduction. Resistance plasmids (R plasmids) defend against environmental threats, while Col plasmids eliminate competing bacteria. Degradative plasmids assist in breaking down uncommon compounds, showcasing microbial adaptation. Virulence plasmids transform bacteria into pathogens, causing illness. Conjugative plasmids enable genetic material transfer, while non-conjugative plasmids rely on assistance during conjugation. Plasmid profiling aids bacterial identification through banding patterns, a powerful method involving digestion and agarose gel electrophoresis.

Basic principle of plasmid DNA isolation

The underlying principle of plasmid DNA isolation hinges on the physical differences between chromosomal DNA and plasmids, primarily their size. Notably, the largest plasmid in E. coli constitutes only 8% of the chromosomal DNA, underscoring the importance of size as a differentiating factor.

Further differentiating chromosomal DNA and plasmids is their conformation, plasmids adopting a circular form while chromosomal DNA exhibits a linear conformation. During the extraction process, chromosomes may break down, forming linear fragments.

The separation of plasmid DNA based on size requires careful consideration, as larger fragments of chromosomal DNA are more easily distinguishable than smaller, broken fragments.

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Published: 06 Feb. 2024
ISSN: 2583-7850

There are mainly two methods for effective plasmid DNA separation i.e., Boiling method and Alkaline lysis method.

Boiling method

The boiling lysis method is a rapid approach for isolating small plasmids (up to 10 kb). For larger plasmids exceeding 10 kb, it is recommended to use alternative methods such as alkaline lysis for better-quality results.

In the boiling method, STET solution (containing sodium chloride, Tris-HCl at pH 8.0, EDTA, and Tween 20) is added to an E. coli pellet. The sample is heated to 100°C for 1 minute, followed by high-speed centrifugation. Plasmid DNA is recovered in the solution, with heat-denatured proteins forming debris as a pellet. The isolated plasmid DNA is then precipitated with alcohol, producing a final sample ready for subsequent experiments like enzymatic cutting or DNA modification.

Alkaline lysis method

Introduced by Birnboim & Doly in 1979, the alkaline lysis method efficiently extracts plasmids from bacterial cultures. Bacteria with the target plasmid are harvested, suspended, and lysed in an alkaline solution containing SDS and NaOH. The detergent facilitates cell lysis and protein denaturation, while the alkaline conditions induce denaturation of genomic and plasmid DNA. The alkaline lysate is then neutralized with potassium acetate, triggering the renaturation of plasmid DNA. High-speed centrifugation separates genomic DNA precipitate, leaving a plasmid-rich supernatant. Plasmid DNA is recovered through precipitation using isopropanol or ethanol, ensuring purified isolation.

Purification of soluble plasmid DNA

Various methods exist for purifying soluble plasmid DNA. One common approach involves precipitating DNA with ethanol or isopropanol. Alternatively, high salt solutions like ammonium acetate, lithium chloride, sodium chloride, or sodium acetate can induce DNA precipitation, effectively separating plasmid DNA from other cellular components.

Another method involves binding plasmid DNA to solid supports such as fibers, glass, or silica. This process allows contaminants to be washed away, resulting in purified plasmid DNA suitable for various molecular biology applications. The choice of methods depends on the specific experiment's needs.

Electrophoretic separation

An effective method widely used for diverse aspects of plasmid DNA analysis is electrophoresis. It enables the detection of plasmid DNA presence in a sample and differentiation between various plasmid species based on their unique migration patterns. Additionally, electrophoresis helps determine the molecular weight of separated plasmids, offering insights into

Published: 06 Feb. 2024
ISSN: 2583-7850

their structural characteristics. The technique also aids in estimating the approximate copy number of plasmids by assessing band intensity, making it a versatile tool for comprehensive analysis and characterization of plasmid DNA in various molecular biology applications.

Determination of yield

Determination of DNA concentration commonly achieved through a combination of UV spectrophotometry and quantitative analysis on an agarose gel. Plasmid DNA is typically diluted in TE buffer at ratios of 1:100 or 1:50, depending on the plasmid copy number, for UV spectrophotometry. The diluted sample is then measured for absorbance at 260 nm and 280 nm, offering valuable insights into nucleic acid concentration. This dual assessment method, incorporating UV spectrophotometry and agarose gel analysis, enhances the precision of DNA concentration determination in various molecular biology applications.

Application of plasmid profiling

Plasmid profiling plays a pivotal role across various applications in molecular biology. One notable application lies in its contribution to understanding antibiotic resistance mechanisms. Plasmids also act as vectors, serving both as expression vectors and cloning vectors, facilitating gene cloning. Additionally, they play a crucial role in recombinant protein production and are integral to gene therapy strategies. Plasmid profiling is also employed to investigate numerous bacterial diseases, aiding in the exploration of their genetic makeup and potential treatment options. Furthermore, plasmids are used for the artificial and cost-effective bulk production of antibiotics. This is achieved by incorporating expression vectors specific to the desired antibiotic into microbial cells, offering a versatile approach for enhancing antibiotic production in a controlled and efficient manner.

Conclusion

Plasmids, genetic elements known for their versatility. They possess a vast range of genes, including those that confer resistance to poisons, antibiotics, and other adaptive features. Bacterial populations evolve as a result of the quick spread of genetic information by horizontal transfer of plasmid. Plasmid profiling offers a window into this dynamic exchange, providing insights into the mechanisms driving bacterial adaptation and survival. The diverse applications of plasmid profiling underscore its significance in advancing research and applications across various domains within the field of molecular biology.

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Published: 06 Feb. 2024
ISSN: 2583-7850

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ISSN: 2583-7850