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Transformation

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ARTICLE ID: 57

Abstract

They developed a method to improve the transformation efficiency of genome-sequenced bacteria using 'plasmid artificial modification' (PAM) using the host's own restriction system. In this method, the shuttle vector was premethylated in E. coli cells carrying all putative genes encoding her DNA-modifying enzymes in the target microorganism before electroporation. Introduction of the DNA methyltransferase gene increased transformation efficiency by 5 orders of magnitude with type II restriction enzymes, and this concept could also be applied to type I restriction systems. This method has been used to transform bacterial cells with higher efficiencies and has also been used to extract intact plasmids from transformed cells with efficiencies comparable to conventional alkaline lysis or CsCl equilibrium density gradient methods. It has been. This technique is simple and rapid, allowing the transformation or production of microgram quantities of plasmid in minutes.

Introduction

A large amount of sequence information on bacterial genomes has become available. To date, 670 bacterial whole genome sequences have been published and over 1900 projects are underway. However, much of the data has been inefficiently used in molecular biology studies due to the unavailability of reverse genetic tools such as convenient shuttle vectors, efficient transformation procedures, gene knockout and random mutagenesis techniques. Therefore, we have been working on the development of a simple method to establish a transformation technology for bacteria whose genome sequence is known. It is well known that most bacteria possess specific restriction-modification systems (R-M) that act as barriers against the entry of foreign DNA, such as via infecting phages or conjugative plasmids. Although there are several methods for gene transfer into eukaryotic systems, introducing

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exogenous DNA into bacteria remains a challenge. However, existing techniques have some limitations and are amenable to further research. This overview attempts to answer basic conversion questions and provides the rationale, requirements, and available options.

What is transformation?

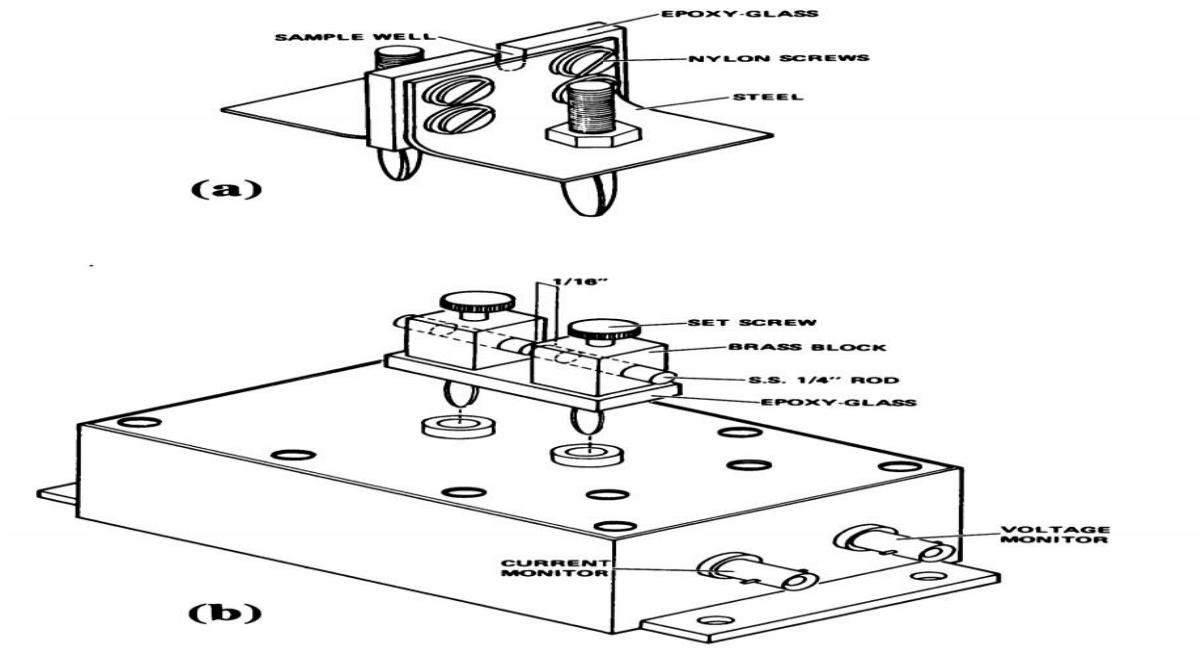
Transformation is the process of transferring exogenous genetic material from a source into a suitable host, preferably a bacterium. It is one of the methods of horizontal gene transfer alongside conjugation and transduction. Exogenous DNA is sometimes referred to as the environment-pure DNA that is transferred to the bacterial host.

Natural transformation

The process by which bacteria can take up and integrate exogenous free DNA from the environment is called natural transformation into bacteria. The importance of natural transformation lies in ecological, evolutionary, and adaptive aspects, and such horizontal gene transfer naturally drives genetic variation and even evolution of virulence factors. Nature's abilities are also used as genetic tools.

Electroporation

The incompetence of most bacterial strains has given rise to alternative methods of bacterial transformation using electrical current. The principle lies in the distortion of the membrane that allows uptake of exogenous DNA. The formation of small local holes by electric currents aids the transformation process.



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Originally used in eukaryotes, it has long been used in the process of bacterial transformation. Expensive and commercially viable. The electro oration process is controlled by the apparatus using a capacitor discharge device that generates exponentially decaying pulses at electric field strengths between 125 and 6250 V/cm. The buffer conductivity and capacitor selection determine the pulse duration, and the voltage is gradually adjusted between 50 and 2500 V. Lakshmi Prasana and others presents a detailed description of the principles and applications of electro oration, along with practical considerations.

Micro-Shock Wave

The limitations of electro oration in terms of cost and other technical capabilities have accelerated the development of new techniques, giving rise to microwave-based bacterial transformation methods. However, the method is device-oriented and several prototypes have been designed so far. In the laboratory, micro - shock waves can be generated using pulsed laser beam focusing, electrohydraulic, piezoceramics, and controlled detonation. The application of underwater shock wave generators is also used for bacterial transformation.

Electrospray Technique

Electrospray uses an electric field to disperse and accelerate droplets or micro particles. No pre - treatment of competent cells is required. This principle uses gold nanoparticles bound to DNA. This results in increased plasmid motility and greater cell wall penetration during electrospray.

Conclusion

Bacterial transformation is an inevitable step not only in molecular biology but also in genetic engineering. With advances in genetic engineering technology, there is a wide variety and size range of recombinant DNA that finds a way to insert into the appropriate bacterial host. The lack of potency of most bacterial strains poses challenges for overcoming obstacles.

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