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Techniques and Methodologies of Molecular Biology

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Description

Molecular cloning

A DNA sequence of interest is isolated and then transferred into a plasmid vector using molecular cloning. The technology of recombinant DNA was initially created in the 1960s. A DNA sequence coding for a protein of interest is cloned into a plasmid using Polymerase Chain Reaction (PCR) and/or restriction enzymes (expression vector). A replication origin, a Multiple Cloning Site (MCS), and a selection marker all distinguish the plasmid vector (usually antibiotic resistance). The promoter regions and transcription start site are located upstream of the MCS and influence the expression of the cloned gene.

This plasmid can be used in either animal or bacterial cells. Transfection of DNA into bacterial cells can be accomplished through bare DNA uptake, conjugation *via* cell-cell interaction, or viral vector transduction. Transfection is the process of physically or chemically introducing DNA into eukaryotic cells, such as animal cells. Calcium phosphate transfection, electroporation, microinjection, and liposome transfection are among the various transfection procedures available. The plasmid can either be integrated into the genome for stable transfection or remain independent of the genome for transient transfection.

A cell now has DNA coding for a protein of interest, and the protein can be produced. To help express the protein of interest at high levels, a range of mechanisms, including as inducible promoters and particular cell-signalling factors, are available. The bacterial or eukaryotic cell can then be used to extract large amounts of a protein. The protein can be examined for enzymatic activity in a variety of conditions, crystallised to study its tertiary structure, or the activity of new medications against the protein can be researched in the pharmaceutical sector.

Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction (PCR) is a very versatile DNA copying technology. In a nutshell, PCR allows a specified DNA sequence to be replicated or changed. The reaction is incredibly potent, and under ideal conditions, one DNA molecule may be multiplied by 1.07 billion in less than two hours. The study of gene expression, the identification of harmful microbes, the detection of genetic mutations, and the introduction of mutations into DNA are all applications of PCR. The PCR technique can be used to add restriction enzyme sites to the ends of DNA molecules or to modify specific bases of DNA, a process known as site-directed mutagenesis. PCR can also be used to see if a certain DNA fragment is present in a cDNA library. PCR comes in several forms, Including Reverse Transcription PCR (RT-PCR) for RNA amplification and, more recently, quantitative PCR for measuring DNA or RNA molecules quantitatively.

Gel electrophoresis

Gel electrophoresis is a technique that uses an agarose or polyacrylamide gel to separate molecules based on their size. One of the most important instruments in molecular biology is this approach. The basic premise is that by passing an electric current through an agarose gel, DNA fragments can be separated; because the DNA backbone contains negatively charged phosphate groups, the DNA will migrate through the gel towards the positive end of the current. Proteins can also be separated by size using an SDS-PAGE gel, or by size and electric charge using a 2D gel electrophoresis.

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