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Editorial Note

Development Editor Note: Molecular Biology Techniques

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Molecular cloning is one of the most basic techniques of molecular biology to study protein function. Polymerase chain reaction (PCR), and/or restriction enzymes into a plasmid (expression vector) are used in this technique to clone a DNA coding for a protein of interest. A desired DNA to be cloned which is obtained from an organism of interest in a conventional molecular cloning experiment, then to generate smaller DNA fragments treated with enzymes in the test tube. Subsequently, to generate recombinant DNA molecules these fragments are then combined with vector DNA. This recombinant DNA is now injected into a host organism (typically an easy-to-grow, benign, laboratory strain of E. coli bacteria). These recombinant DNA molecules are replicated along with the host DNA which will generate a population of organisms. As they contain foreign DNA fragments, these are called transgenic or genetically modified microorganisms (GMO).

A vector contains an origin of replication, a selective marker and a multiple cloning site (MCS), usually antibiotic resistance. The promoter regions and the transcription start site which regulate the expression of cloned gene are located upstream of the multiple cloning site. Now this plasmid can be inserted into either bacterial or animal cells. Transformation is one method of Introducing DNA into bacterial cells via uptake of naked DNA, cell-cell contact via conjugation or by viral vector via transduction. Introducing DNA into eukaryotic cells (animal cells) by physical or chemical means called Transfection. There are many different transfection techniques are present, such as calcium phosphate transfection, electroporation, microinjection and liposome transfection. The transient transfection is which plasmid may be integrated into the genome, resulting in a stable transfection, or may remain independent of the genome.

Polymerase chain reaction (PCR) is an extremely versatile technique for amplifying DNA. PCR allows a specific DNA sequence to be amplified or modified in predetermined ways. In less than two hours it amplifies one DNA molecule to become 1.07 billion molecules as this reaction is extremely powerful and under perfect conditions to amplify. The restriction enzyme sites to ends of DNA molecules, or to mutate particular bases of DNA, the latter is a method referred to as site-directed mutagenesis is introduced by PCR technique. Whether a particular DNA fragment is found in a cDNA library is also found by PCR. There are many variations in PCR, like reverse transcription PCR (RT-PCR) for amplification of RNA, and, quantitative PCR which allow for quantitative measurement of DNA or RNA molecules more recently.

Gel electrophoresis is another technique which is used to separate and purify macromolecules like nucleic acids and proteins which differ in size and charge. The purpose of Gel electrophoresis is to separate DNA, to separate the size of DNA, RNA, protein, and to purify DNA, RNA, proteins. It involves electric field to separate these RNA, DNA, and proteins through an electrically charged agarose gel and this is called agarose gel electrophoresis. SDS-PAGE gel is which the Proteins can be separated on the basis of size and their electric charge it is known as a 2D gel electrophoresis.