Site Directed Mutagenesis Techniques

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Abstract

Mutagenesis refers to the process of inducing changes or mutations in the genetic material, typically DNA, of an organism. Site-directed mutagenesis is a versatile molecular biology technique essential for the precise manipulation of DNA sequences. This method allows researchers to intentionally introduce specific changes, such as point mutations, insertions or deletions, at predetermined locations in a gene. The key to site-directed mutagenesis is the use of synthetic oligonucleotides, short segments of DNA designed to contain the desired sequence changes. These oligonucleotides serve as primers in a PCR-style reaction, allowing selective amplification of the mutated DNA sequence. The applications of site-directed mutagenesis are broad and powerful. By making targeted genetic modifications, researchers can study the functional consequences of specific mutations, thereby revealing the complexity of gene function. This technique is especially useful in structure-function studies, where researchers want to understand how changes in DNA sequence translate into changes in protein structure and activity. Additionally, site-directed mutagenesis plays an important role in the development of therapeutic agents, allowing scientists to modify genes to improve protein expression or alter biological activity. Overall, site-directed mutagenesis allows scientists to dissect the complexity of biological systems, contributing to advances in biotechnology, medicine, and their fundamental understanding. about cellular processes. The precision and control afforded by this technique make it an indispensable tool for researchers seeking to elucidate the relationship between genetic information and phenotypic outcomes.

Key Words: DNA sequences, Mutagenesis, Phenotypic outcomes, PCR-style.

Introduction

Site-directed mutagenesis is a technique designed in the 1970s and used to mutate specific DNA sequences invitro. It relies on short, single-stranded synthetic DNA fragments, or oligonucleotides, containing mutations designed to act as templates in the presence of the DNA polymerase enzyme. In the late 1980s, Polymerase Chain Reaction (PCR), another laboratory technique, was introduced into site-directed mutagenesis. There are several techniques/ methods to perform site-directed mutagenesis such as;

Overlap extension PCR (OE-PCR) is a two-step PCR technique used to introduce mutations into a DNA sequence. First, two separate PCR reactions were performed. In a reaction, the 5' end of the target DNA (upstream of the mutation site) is amplified using a forward primer and a reverse primer containing the desired mutation. In another reaction, the 3' end of the target DNA (at the end of the mutation site) is amplified using a different set of primers. It is important to note that the primers for each reaction contain overlapping complementary sequences corresponding to the mutation site. OE-PCR is versatile and can be used to generate many different types of mutations, including point mutations, insertions, and deletions. It allows precise control of mutation localization and is cost-effective as it does not require specialized enzymes or reagents. The two-step process ensures that mutations are correctly incorporated (An et al., 2005).

QuikChange PCR is a simple technique for introducing point mutations into a DNA sequence. The method includes a standard PCR reaction with a DNA sample containing the target gene, an additional primer pair, high-fidelity DNA polymerase enzyme, and dNTP. Primers are designed to contain the desired mutation(s) in the middle of their sequence. In the PCR reaction, the primers hybridize to the target DNA at the site of the mutation, and DNA polymerase synthesizes a new DNA strand, incorporating the mutation(s) as it extends.

QuikChange PCR is known for its speed, efficiency and reliability in introducing point mutations. It does not require complicated procedures or expensive reagents, making it a widely used method for point mutagenesis (Hogrefe et al., 2002).

Gene synthesis mutagenesis is a powerful method that involves chemically synthesizing an entire gene from scratch. During the gene design phase, researchers can identify desired mutations in the gene sequence. DNA synthesis machines assemble nucleotides in a designed sequence, creating a synthetic gene. The synthetic gene can then be cloned into a vector for further experiments. Gene synthesis provides complete control over gene sequences, allowing for precise, personalized modifications. It is suitable for creating multiple mutations, complex genetic designs, and creating entirely new genes. However, it can be more expensive than other methods (Ling et al., 1997).

Cassette mutagenesis it involves inserting a piece of DNA (cassette) containing the desired mutation(s) into the target gene. First, the target gene is cut at specific locations using restriction enzymes to create compatible ends. The cassette, which also has compatible ends, is effectively tethered to sites that cut, replace, or alter the gene sequence. Cassette mutagenesis is useful in introducing larger changes, such as insertions or deletions, into genes. It can also be used to add functional elements such as tags or promoters to the gene of interest (Wells et al., 1985).

Restriction enzyme-based mutagenesis this method relies on the specificity of restriction enzymes to create mutations. Researchers choose specific restriction enzymes based on their recognition sequence in the target gene. The DNA is then cut at these specific locations. After digestion, DNA can be repaired by ligation, which often produces mutations or changes in the cut site. Restriction enzyme-based mutagenesis allows precise, site-specific modifications of DNA sequences by exploiting the specificity of restriction enzymes (Horton et al., 1997).

CRISPR-Cas9-based mutagenesis CRISPR-Cas9 is a genome editing tool that can be programmed to target a specific DNA sequence within a gene of interest. The Cas9 protein creates double-strand breaks in the DNA at the target site. When these breaks are repaired by cellular machinery, errors can occur, leading to local mutations. CRISPR-Cas9 provides exceptional precision and specificity in generating mutations. It can be used in cells and living organisms for precise gene editing (Tuladhar et al., 2019).

Oligonucleotide-directed mutagenesis is a technique that uses synthetic oligonucleotides containing the desired mutation(s). These oligonucleotides are used as primers in PCR reactions or primer extension reactions with target genes. During DNA synthesis, oligonucleotides act as templates, leading to the generation of desired mutations. This method is quick and effective in introducing point mutations or making small changes in the DNA sequence(Sauer et al., 2016). The choice of method depends on the specific mutation(s) introduced, the complexity of the gene, the level of control desired, and the resources and expertise available in the laboratory. Researchers choose the most suitable method according to their experimental goals and limitations.

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