Polymerase Chain Reaction

Introduction

The polymerase chain reaction is a revolutionary method developed by Kary Mullis in the 1980s. PCR is based on the ability of DNA polymerase to synthesize new strands of DNA complementary to the template strand. PCR is used for Since DNA polymerase can only add a nucleotide onto a preexisting 3' -OH group, it needs an RNA primer to begin synthesizing nucleotides. Due to this limitation, it is possible to describe a specific region of the template sequence that the researcher wants to amplify. This result of the PCR reaction is the specific sequence will be accumulated in billions of copies, known as amplicons.

Steps of PCR

1. The DNA sample to be amplified is heated to a high temperature, typically around 95°C, to denature the double-stranded DNA into single strands.
2. The DNA sample is cooled in the presence of two nucleotide primers. The primers are complementary to each 3' end of the DNA fragment to be amplified. The low temperature allows the primers to solidify with the 3' ends of the single-stranded DNA to be amplified.
3. The DNA sample is heated to 72°C, which is the optimal temperature for Taq polymerase. The isolation of the DNA polymerase from the Thermus aquaticus was one of the key factors that made PCR possible. Taq polymerase synthesizes DNA by the addition of free nucleotides to the ends of the primers through complementary base pairing. However, unlike other DNA polymerases, it can withstand high temperatures.
4. DNA cycles through steps 1-3 several times. The amount of DNA doubles with each replication cycle. Thus, two copies are produced after the first cycle, four copies after the second cycle and so on.

Fig 1. Stages in PCR

Components of PCR

1. DNA template: The sample of DNA that contains the target sequence. At the beginning of a cycle, high temperature denature the double-stranded DNA molecule
2. DNA polymerase: Enzyme that synthesizes strands of DNA complementary to the template DNA. The first and most commonly used is the *Taq DNA* polymerase whereas *Pfu*DNA polymerase is used widely due to its higher fidelity when copying DNA. Both of the enzymes have capabilities that make them suitable for PCR since they can generate new strands of DNA, and they are heat resistant.
3. Primers: Short pieces of single-stranded DNA that are complementary to the target sequence. The polymerase begins synthesizing new DNA from the end of the primer
4. Nucleotides (deoxynucleotide triphosphates): Single units of the bases A, T, G, and C, which are essentially the building blocks of DNA strands. Reverse Transcription PCR is PCR preceded with the conversion of sample RNA reverse transcriptase into cDNA with enzyme

Limitations to PCR

The PCR reaction generates copies of the target sequence exponentially. Only during the exponential phase of the PCR reaction is it possible to extrapolate back to determine the starting quantity of the target sequence from the sample. Because of inhibitors of the polymerase reaction found in the sample, reagent limitation, which is the accumulation of pyrophosphate molecules, and self-annealing of the accumulating product, the PCR reaction eventually ceases to amplify the target sequence at an exponential rate and it peaks, making the endpoint quantification of PCR products unreliable.

Transgenic bacteria in pharmaceuticals

Pharmaceuticals

The use of living, genetically modified bacteria as delivery vehicles for biologists is of considerable interest scientifically and has attracted significant commercial investment. Most of the hosts used to produce 151 recombinant pharmaceuticals so far approved for human use by the FDA and/or by the EMEA are microbial cells, either bacteria or yeast. Diabetes is a disease caused by excessive amounts of blood glucose. This condition is fatal if untreated and leads to various symptoms such as excessive urination, fatigue, weight loss, and blurred vision. To control blood glucose levels, a hormone known as insulin is made by the pancreas to signal cells the uptake of blood glucose. Type I diabetes is caused by insufficient blood insulin. In 1921, Dr. Frederick Banting researched a successful way to isolate insulin from animals such as dogs and pigs, saving many human lives. However, impurities in the insulin from animals led to allergic reactions. In 1993, Health Canada approved the sale of human insulin produced by transgenic bacteria. Although insulin was purified from animals and produced many adverse side effects in patients these side effects are not present when patients use human insulin produced in bacteria.

Transgenic plants

Introduction

Plant transformation is the process by which DNA is introduced into plant cells or tissues. The DNA can come from virtually any source. Gene transfer methodology has become part of an essential technology to manipulate plants for both scientific and commercial purposes. Historically, it has involved the slow and meticulous process of crossbreeding different varieties to produce plants with desired traits. However, the development of recombinant DNA technology has not only reduced the work and time required to develop new varieties of plants but has also increased the scope of potential biotechnological applications.

GMOs

Agricultural plants are one of the most prominent examples of GMOs. Examples of transgenic crops that have been developed and approved for human consumption include corn, soybeans, tomatoes, and potatoes. The benefits of genetic engineering of crops include increased crop yield, reduction in harvesting cost and pesticide use, and enhanced nutritional value.

Techniques

The two major techniques for introducing foreign DNA into plant cells to produce transgenic plants are the **biolistic method** and **the Ti plasmid method**. The biolistic method involves the striking of plant cells with tiny particles of gold or platinum that are coated with DNA. The bombardment occurs at a very high speed, which allows the DNA to penetrate the cell wall of plant cells. However, this method lacks control concerning the insertion site of the gene and how many copies are introduced. Many biotechnologists argue against using this method because the introduced gene may insert into a functional gene, potentially altering that gene's function.

The Ti plasmid method uses the same principles as the production of transgenic bacteria. The Ti plasmid occurs naturally in the bacterium *Agrobacterium tumefaciens*. These bacteria naturally infect plant cells and cause the formation of a bulbous growth on the plant. Part of the Ti plasmid (T-DNA) integrates into the plant genome and causes uncontrolled cell growth, which results in a tumour. Researchers have altered the T-DNA of the Ti plasmid so that it can no longer cause tumour formation but still allows for the integration of DNA into the plant genome.

Gene pharming

Gene pharming is the production of pharmaceuticals by using animals or plants that have been genetically engineered. Pharming is a useful alternative to traditional pharmaceutical development because genetically engineered livestock and plants are relatively inexpensive to produce and maintain. Genetic pharming remains controversial due to concerns about the safety of pharmed agents and their production. It also involves protein production in the mammary glands of the transgenic animal. The human protein is excreted into the animal's milk, the milk is collected, and the protein is purified from it. A foreign protein is targeted to mammary cells through the use of a β-lactoglobulin promoter in the recombinant DNA containing a gene that encodes for the protein. B-lactoglobulin is a protein that is expressed in mammary cells of many animals, such as animals like cows and sheep.

Mammalian Cloning

Several mammalian species have been cloned by transferring nuclei from various adult somatic cells into enucleated oocytes. However, the cloning procedure is still inefficient since only one in a hundred manipulated oocytes develops to adulthood. There are several reasons for the low

efficiency of cloning, but the biggest factor is the incomplete or incorrect reprogramming of the donor nuclear genome.

Fig 5: Process of mammalian cloning

Bacterial Transformation

Bacteria transformation is the mechanism in which the targeted gene is uptaken by a bacterium, the plasmid which acts as a vector. The plasmid has selectable markers such as genes for antibiotic resistance and the lacZ gene. An interesting gene is inserted into the plasmid so that it disrupts the lacZ gene to make it inactive. The bacteria are cultivated on Petri dishes, where they can formulate colonies and can be easily identified by a reaction occurring with the selectable marker and the external environmental factors. The transformed bacteria can then be transferred to a liquid culture to be amplified or can be used immediately following the transformation process in a transgenic manner

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