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Science, Genetics

Polymerase Chain Reaction

Polymerase chain reaction known as PCR was developed by Kary Mullis in 1983. It is a Molecular Diagnostic technology used for amplification of DNA. A single copy of DNA can be amplified to higher magnitudes with a thousand copies of a single DNA sequence. PCR has now been developed as a Molecular Diagnostic tool for Cancer and mainly to detect the early onset of leukemia or lymphomas. It is widely used as a diagnostic tool in the field of microbiology and is also utilized in detection of infectious micro-organisms y detecting specific disease causing genes. In the year 1993, Mulis was awarded the Noble prize for work done on PCR. The technique of PCR however requires certain components and strict temperature control for proper amplification and analysis of a Particular gene of interest.

Principles of PCR (PCR, National Institute of Health website. Fact files)

The basic principle underlying PCR is amplification of a single sequence or strand of DNA. DNA sequence length can vary from a mere 0. 1kilo base pairs (kb) to a 1000 kb. However in PCR, the maximum length used for amplification is around 40kb. The PCR method is quite simple and quick. It can amplify DNA from small quantities of up to 0. 1kb and can also amplify DNA from damaged samples. Although mentioning amplification several times, the concept lies in when a single molecule of DNA is converted to two, four, and eight and so on, thus implying a chain reaction. This is done with the help of specific enzymes called Polymerases. These help DNA to elongate into long strands. However polymerases do need building blocks of DNA,

termed as Nucleotides. The nucleotides are termed as Adenine (A), Thymine (T), Cytosine (C) and Guanine (G). Additionally a primer is needed for constructing of new strand of DNA. All these requirements are essential for successful amplification of a DNA sequence. It takes around 2 hours for the reaction and can be visualized by gel electrophoresis. (Joshi et al)

Components and Procedure

Carrying out PCR may often require different temperature controls. Maintaining temperature is crucial and can be done through an automatic sequencer which enables cooling and heating of coils. This automated system makes it easier for the sample to be in a single place preventing any kind of human or technical error. However the main components for carrying out the chain reaction include a DNA template. This DNA template contains the sequence of interest and is subjected to High temperature initially (Separation of strands). The next major component requires is Enzyme Polymerase. It helps in synthesis of a new DNA strand. A classic example of DNA polymerase is Taq polymerase. It is obtained from Thermis aquaticus. The major advantage of this enzyme is its ability to synthesize new DNA strands and also its resistance to heat. Although enzymes and polymerase are essential, but without the building blocks of DNA, amplification of selected sequence is not possible. As mentioned earlier, the four nucleotides Adenine, Thymine, Guanine and Cytosine are required. Once all the components for PCR are acquired, the reaction can be carried in three basic steps as follows: (NIH Website Fact files)

Denaturation: This step involves denaturation or separation of the selected

DNA sequence. It is carried out at 94ºc for around half a minute. During this step the DNA double strand opens up into two distinct single strands, which form templates for amplification.

Annealing: Primers are in the solution and move about to get attached to the single stranded DNA. The temperature is lowered to 55ºc for annealing of the primer to the single stranded DNA template. Also the DNA polymerase attaches to the template and begins with the formation of new DNA strand. The temperature is maintained for around 20 to 40 seconds. (George J. Netto et al. 2003)

Elongation: The temperature is slightly raised to around 72ºc, this temperature is considered ideal for Polymerase to work. Primers with strong ionic bonding, bind with the DNA template and those with weak binding forces get separated. Activity of Taq polymerase is highest at 72ºc and it polymerizes a new DNA strand complimentary to the DNA template. The elongation procedure is dependent on the polymerase used and the length of DNA strand to be amplified. (George J. Netto et al. 2003)

The following steps are carried out 20 to 40 times depending on the specific gene of interest. However to be more technical, 20 to 40 cycles are carried out per reaction in order to get accurate and precise results. The end product can be visualized using gel electrophoresis. (Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology.)

Comparing PCR with other Diagnostic Techniques

PCR is the most widely accepted diagnostic technique for quantification of specific DNA sequences. This method allows researchers to detect any gene

mutation or specific detection and quantification of a gene of interest. (Gene maybe of importance in immunology, microbiology and genetics). PCR can be directly compared to its counterpart Real Time RT-PCR. RT-PCR enables the detection of amplification during the process of reaction. It can also measure kinetics of amplification compared to traditional PCR. There is no use of Gel electrophoresis at the end of the reaction since it is detected in real time through advanced computerized imaging and technology. RT-PCR detects and collects data in exponential growth phase of Polymerase reaction. Use of fluorescence enables easy and accurate detection of desired amplicons (PCR products). There is no post-treatment or post-analysis in RT-PCR method. Detection rate is quite higher compared to traditional PCR.

Strengths of Polymerase Chain Reaction

The main advantage of PCR is its high sensitivity. Another main reason is amplification of DNA strand from a single sequence of DNA. Damaged or very small quantities of starting DNA material can be used for amplification. (PCR, University of Colorado)

Limitations of Polymerase Chain Reaction (PCR)

PCR is used for quantification of desired DNA template. However the use of end-point techniques like agarose gel electrophoresis might have many disadvantages. It is said to have low precision and low in sensitivity. Traditional PCR methods are not automated and hence improper quantification of DNA is possible due to improper temperature maintenance. Also it is said to have a very low resolution compared to other advanced techniques of PCR like RT-PCR. Traditional PCR also has a major

disadvantage of size discrimination in amplification process. The use of Ethidium bromide is not quantitative and is also a potent carcinogen. Post reaction process is time consuming and low in accuracy and precision. Overall PCR may encounter many issues in quantification and precision, but it is somehow still used in most laboratories. However an automated sequencer is used to combat the temperature differences.
(Real-Time PCR Vs. Traditional PCR, Applied Bio-systems)

Applications

PCR is used in broad range of industries and Clinical Laboratories. The main purpose of PCR is diagnosis of diseases. It is used in Viral Gene and gene expression quantification respectively. Different assays can be verified using PCR. Detection of any damage to DNA strand is possible through PCR. In clinical trials PCR is used to validate drug efficacy. PCR is used by microbiologists in detection of pathogenic strains. It is also used in genotype detection. (Real-Time PCR Vs. Traditional PCR, Applied Bio-systems)

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