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POLYMERASE CHAIN REACTION

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Abstract*. The method is based on the detection of even small concentrations of the desired diagnostic element. To determine the initially extremely small concentrations of RNA or DNA that need to be determined during the main stage of the study, a method is used to artificially increase the amount of RNA or DNA.*

Keywords*: PCR, RNA, DNA, replication, polymerase, Recombinase Polymerase Amplification, Transcription, genetic fingerprints, asymmetric PCR, Reverse Transcription PCR, Inverse PCR.*

Relevance.

The method is based on repeated selective copying of a certain section of DNA nucleic acid using enzymes under artificial conditions (in vitro). In this case, only the section that satisfies the specified conditions is copied, and only if it is present in the sample under study. Unlike DNA amplification in living organisms (replication), PCR amplify relatively short sections of DNA. In a conventional PCR process, the length of the copied DNA sections is no more than 3000 base pairs (3 kbp). Using a mixture of various polymerases, using additives and under certain conditions, the length of a PCR fragment can reach 20-40 thousand nucleotide pairs. This is still significantly less than the length of the chromosomal DNA of a eukaryotic cell. For example, the length of the shortest nuclear chromosome in humans (chromosome 21) is 46.71 million base pairs.

To carry out PCR in the simplest case, the following components are required:

A DNA template containing the portion of DNA that needs to be amplified. Two primers complementary to opposite ends of different strands of the desired DNA fragment.

Thermostable DNA polymerase is an enzyme that catalyzes the polymerization reaction of DNA. Polymerase for use in PCR must remain active at high temperatures for a long time, so enzymes isolated from thermophiles are used - Thermus aquaticus (Taq polymerase), Pyrococcus furiosus (Pfu polymerase), Pyrococcus woesei (Pwo polymerase), Thermus thermophilus (Tth polymerase) and others. Deoxyribose[fr]nucleoside triphosphates (dATP, dGTP, dCTP, dTTP). Mg2+ ions necessary for the polymerase to function.

A buffer solution that provides the necessary reaction conditions - pH, ionic strength of the solution. Contains salts, bovine serum albumin. To avoid evaporation of the reaction mixture, add high-boiling oil, such as Vaseline, to the test tube. If you are using a thermal cycler with a heated lid, this is not required.

The addition of pyrophosphatase can increase PCR yield. This enzyme catalyzes the hydrolysis of pyrophosphate, a byproduct of the addition of nucleoside triphosphates to the growing DNA strand, to orthophosphate. Pyrophosphate may inhibit PCR

Materials and methods of research.

RPA (Recombinase Polymerase Amplification) is used where DNA/RNA amplification is required within 15 minutes without a thermal cycler (isothermal reaction).

Nested PCR is used to reduce the number of reaction byproducts. Two pairs of primers are used and two sequential reactions are carried out. The second pair of primers amplifies a region of DNA within the product of the first reaction.

Inverse PCR (inverse PCR) - is used if only a small region within the desired sequence is known. This method is particularly useful when it comes to determining neighboring sequences after DNA has been inserted into the genome. To carry out inverted PCR, a series of DNA cuts with restriction enzymes are carried out, followed by joining of fragments (ligation). As a result, known fragments end up at both ends of the unknown region, after which PCR can be carried out as usual.

Reverse Transcription PCR (RT-PCR) - used to amplify, isolate, or identify a known sequence from an RNA library. Before conventional PCR, a single-stranded DNA molecule is synthesized on an mRNA template using reversease and a single-stranded cDNA is obtained, which is used as a template for PCR. This method is often used to determine where and when these genes are expressed.

Asymmetric PCR (asymmetric PCR) - is carried out when it is necessary to amplify predominantly one of the strands of the original DNA. Used in some sequencing and hybridization analysis techniques. PCR is carried out as usual, except that one of the primers is taken in large excess. A modification of this method is Linear-After-The-Exponential-PCR (LATE-PCR), in which primers with different concentrations are used, and the low concentration primer is selected to have a higher (melting point) than the high concentration primer. PCR is carried out at high annealing temperatures, thereby maintaining the efficiency of the reaction throughout all cycles

The purpose of this study

Forensics

PCR is used to compare so-called “genetic fingerprints.” A sample of genetic material from the crime scene is required - blood, saliva, semen, hair, etc. This is compared with the genetic material of the suspect. A very small amount of DNA is enough, theoretically one copy. The DNA is broken down into fragments and then amplified using PCR. The fragments are separated using DNA electrophoresis. The resulting pattern of the arrangement of DNA bands is called a genetic fingerprint.

Establishing paternity

Although genetic fingerprints are unique, relationships can still be established by making multiple fingerprints. [≡] The same method can be used, with slight modifications, to establish evolutionary relatedness among organisms.

Medical diagnostics (PCR test)

PCR makes it possible to significantly speed up and facilitate the diagnosis of hereditary and viral diseases. The gene of interest is amplified by PCR using appropriate primers and then sequenced to identify mutations. Viral infections can be detected immediately after infection, weeks or months (depending on the incubation period) before symptoms appear.

Personalized medicine

Sometimes medications turn out to be toxic or allergenic for some patients. The reasons for this are partly due to individual differences in the susceptibility and metabolism of drugs and their derivatives. These differences are determined at the genetic level. For example, in one patient

a certain cytochrome (a liver protein responsible for the metabolism of foreign substances) may be more active, in another - less active.

Conclusion.

PCR is a simple and widely used process in which minute amounts of DNA can be amplified into multiple copies. In addition to the rapidity with which this assay works, it is able to quantitatively demonstrate how much of a particular sequence is present. As with all methods, the validity of the results should be compared with the specificity associated with the method. The future of PCR is promising, combining various assays and approaches to produce greater insight into various gene combinations. For example, in a study to link distinct taxa within the microbial community to specific metabolic processes, stable isotope probing was combined with qPCR. Microarray experiments can be validated by qPCR approaches due to its rapidity.

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