Volume 2, Issue 6, June 2024 ISSN (E): 2938-3765

PCR METHOD OF MODERN LABORATORY DIAGNOSTICS

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Abstract
Polymerase chain reaction (PCR) is an experimental method in molecular biology that can achieve significant increases in small concentrations of certain nucleic acid (NA) fragments in biological material.

Keywords: polymerase chain reaction, nucleotides, viruses, phosphatic acid, chain.

Introduction

The discovery of the polymerase chain reaction was preceded by the development of molecular biological technologies. DNA is a polymeric molecule consisting of repeating blocks - nucleotides. Each nucleotide consists of a nitrogenous base, a deoxyribose and a phosphate group. The bonds between nucleotides in the chain are formed by deoxyribose and a phosphate group. In the vast majority of cases (except for some viruses containing single-stranded DNA), the DNA macromolecule consists of two strands oriented with nitrogenous bases toward each other. Four types of nitrogenous bases (adenine, guanine, thymine, and cytosine) are found in DNA [1,2].

The nitrogenous bases of one of the chains are connected with the nitrogenous bases of the other chain by hydrogen bonds according to the principle of complementarity: adenine is connected only with thymine, guanine - only with cytosine. Each chain serves as a matrix in the synthesis of a new chain, and the sequence in the synthesized chain is given by the sequence of complementary bases of the matrix chain. The asymmetrical ends of the DNA chain are called 3' (three-stranded) and 5' (five-stranded). The polarity of the chain plays an important role in DNA synthesis (chain elongation is possible only by attaching new nucleotides to the free 3'-end). Thus, the principle of

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using PCR as a method of in vitro amplification of specified DNA fragments with fully or partially known sequence was formed. PCR requires the presence of a number of basic components in the reaction mixture [4,6,8,9].

Primers are artificially synthesized oligonucleotides, usually 15 to 30 nucleotides in size, identical (complementary) to the opposite ends of the opposite strands of the target DNA strand. They serve as primers for the synthesis of the complementary strand by DNA polymerase and play a key role in the formation and accumulation of amplification reaction products. Properly selected primers ensure specificity and sensitivity of the test system and must meet a number of criteria:

-**To be specific.** Particular attention is paid to the 3'-ends of primers, since it is from them that Taq polymerase starts to complete the complementary DNA chain. If the specificity of primers is insufficient, products will be formed in the PCR process, which, on the one hand, can be identified as false positives, and on the other hand, their accumulation will consume components of the reaction mixture, resulting in a significant loss of sensitivity of the reaction as such;

-**Must not form** dimers and loops - stable double chains - when primers anneal on themselves or with each other;

-**The primer annealing region should be** outside the zones of mutations, deletions or insertions within the species or other specificity taken as a criterion for primer selection. If the primers fall into such a zone, no primer annealing occurs and, as a consequence, a false-negative result occurs.

Taq-polymerase is a thermostable enzyme that ensures completion of the 3'-end of the second strand of DNA according to the principle of complementarity. A mixture of deoxynucleotide triphosphates (dNTPs) - deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytosine triphosphate (dCTP) and deoxythymidine triphosphate (dTTP) - is the building material used by Taq polymerase for the synthesis of the second strand of DNA [4].

Buffer - a mixture of cations and anions in a certain concentration providing optimal conditions for the reaction and stable pH value. Analyzed sample - a preparation prepared for introduction into the reaction mixture, which may contain the desired NK, for example, DNA of microorganisms, serving as a target for subsequent multiple copying. In the absence of the target DNA, no specific amplification product is formed [2,5].

For convenience of detection and control of amplification efficiency, additional components may be included in the reaction mixture. The positive control sample (PCS) is an artificially synthesized oligonucleotide sequence that strictly corresponds to the target sequence. Accordingly, the primers for the PCS and the desired target are identical, which makes it possible to make sure that the PC components necessary for normal PCR are functional and intact [1,2,3].

The Negative Control Sample (NCS) includes all components of the reaction, but an appropriate amount of deionized water or extract containing no test DNA is added in place of the clinical material or NC preparation. Negative control is necessary to check the reaction components for the absence of DNA or pathogen cells due to contamination and to exclude consideration of false positives. The NK preparation may contain impurities of inhibitors that markedly reduce PCR efficiency, and in some cases may lead to complete absence of results. In addition, there may be

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errors at the stage of PC preparation (e.g., some component or NK itself was not added), non-compliance with the temperature conditions of storage of reagent kits or their individual parts (e.g., thawing and loss of enzyme activity), and a number of other technical issues that directly affect the PCR results. Therefore, it becomes necessary to control the progress of amplification in each tube with the reaction mixture. For this purpose, an additional internal control (IC) is introduced into the MS. An IC is an artificially designed DNA or RNA preparation that has a fundamentally different oligonucleotide sequence from the desired one. For VC, the MS contains its own strictly complementary primers. The concentration of BK in the MS should be such that it does not compete for amplification of even single sought NK molecules [5,7].

The presence of IC amplicons is evidence of normal passage of the amplification reaction. If amplicons of the desired NC are absent, but no amplicons of BK were also formed, we can conclude that there are technological disorders or undesirable impurities in the analyzed sample. In either case, the reaction result should be recognized as unreliable. IC can be used not only directly in the MS for amplification, but also for quality control of NK isolation. For this purpose, it is introduced into each tube with the initial or pre-treated sample, carried through the isolation step and only then added to the MS. The introduction of EC with a known concentration at the isolation step is especially important for controlling quantitative PCR analysis [3,5].

The use of special controls during PCR allows solving a number of problems, primarily concerning the evaluation of the efficiency of the amplification process and control of the specificity of the obtained results, as well as provides an opportunity to realize an approach to quantitative DNA analysis [7,9,11].

It is fundamental to set special controls when studying complex multi-component systems such as biocenoses, since it is possible to qualitatively and quantitatively analyse the interaction of system components and characterise their relationship to the biotope. The following can be referred to special controls:

-DNA fragment length markers;
-background control;
-standards and calibrators;
-material collection control (MCC).

DNA fragment length markers are used in the detection of PCR results by gel electrophoresis. Standards (markers) are fragments of double-stranded DNA of a strictly defined length that allow identification and characterization of bands obtained in the gel and evaluation of the analysis results in terms of their specificity. Background control is used for PCR with fluorescence-based detection methods, and this type of control is most relevant for PCR with endpoint detection. Analysis of the fluorescence levels of the target and background fluorescence allows to establish a certain threshold value and to determine the criteria of reliability of positive and negative results of PCR with endpoint detection. Background control is used for PCR with fluorescence-based detection methods, and this type of control is most relevant for PCR with endpoint detection. Analysis of the fluorescence levels of the target and background fluorescence allows to establish a certain threshold value and to determine the criteria of reliability of positive and negative results of PCR with endpoint detection [10,11,12].

Standards and calibrators are most commonly used in quantitative PCR analysis with real-time detection of results. A standard is an arbitrary fragment of DNA or RNA restricted with the same primers as the target. Known concentrations of the standard correspond to certain values of the

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threshold cycle Ct (the number of the first cycle in which there is an increase in the fluorescent signal of the reporter group compared to the background signal level; its value depends on the initial number of copies of the matrix and on the efficiency of DNA amplification). The introduction of this type of control involves the construction of a calibration graph from which the concentration of the desired NC in the test sample is found. The accuracy of the method depends on how close the PCR conditions of a series of standards (primarily amplification efficiency) are to the PCR conditions of the experimental samples [9,10].

The following standard options exist for determining the amount of matrix in PCR-RV:

-purified PCR-RV product;

-recombinant DNA;

-recombinant RNA followed by reverse transcription;

-synthetic oligonucleotide containing the sequence to be amplified.

The use of standards and calibrators allows the DNA concentration to be determined in two ways (e.g., when analyzing for pathogens in a sample):

-the number of genomic equivalents of microorganism cells per unit volume of clinical sample (GE/mL), which reflects the absolute concentration of these microorganisms in the clinical material;

-calculation of the ratio of the number of genomes to the number of human cell genomes. For this purpose, human DNA calibrators are present in the PCR mixture along with microorganism DNA calibrators. The relative values of microorganism DNA concentration to human DNA obtained in this way can reflect the density of contamination with the desired microorganisms [2,5,8].

Control of material collection is a key point in determining the quality of the sample taken for examination. This approach eliminates pre-analytical errors in the examination of biological material containing human cells and avoids false-positive or false-negative PCR results. In addition, it can be used to estimate the amount of human genomic DNA [1,3].

Thus, there is a spectrum of approaches that provide reliable results that allow to control the quality and efficiency of PCR passage and optimize laboratory performance.

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