Volume 2, Issue 10, October 2024 ISSN (E): 2938-3765

TECHNOLOGY OPTIONS FOR MODERN DIAGNOSTIC METHOD-PCR

Umarova Tamila Abdufattoevna
Assistant of the Department of Clinical Laboratory
Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD;

Kudratova Zebo Erkinovna
PhD, Associate Professor of the Department of Clinical Laboratory
Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD;

Narzullayev Qamariddin Xamdam ogli
Cadet (Laboratory Doctor) of the Department of Clinical Laboratory
Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD;
Samarkand State Medical University Samarkand, Uzbekistan

Abstract
At present, variants of PCR staging have been developed aimed at solving the following problems: increasing the efficiency of the reaction and reducing the risk of non-specific products formation; qualitative and quantitative analysis of the desired regions of the DNA/RNA molecule.

Keywords: modifications of PCR, DNA, RNA, amplification, qualitative and quantitative analysis.

Introduction

The most common modifications of PCR are:

Hot-start PCR - modification of the method aimed at preventing nonspecific interaction of the reaction mixture components until optimal amplification conditions are achieved. Depending on the GC composition and size, primers have a certain melting temperature (Tm is the temperature at which half of the DNA matrix forms a complex with the oligonucleotide primer in the presence of an enzyme - polymerase). Under optimal conditions (primer annealing temperature is close to the melting temperature), the primer forms a stable complementary bond with the matrix. If the temperature of the reaction mixture is below Tm, nonspecific annealing of the primer to the matrix and formation of dimers is possible. If there is no active enzyme in the reaction mixture at the moment, elongation of the formed nonspecific complex does not occur, thus avoiding false positive PCR results. There are two main ways to implement hot start:

1. blocking of polymerase by antibodies or antibody-mimicking small molecules such as Affibody. Polymerase becomes active only when the reaction mixture is preheated at 95 °C for 10 minutes - these are the conditions under which the polymerase-antibody bonds are broken [2,3,4].

Licensed under a Creative Commons Attribution 4.0 International License.

Volume 2, Issue 10, October 2024 ISSN (E): 2938-3765

2. the use of easily melting paraffin separates the polymerase from the components of the reaction mixture, preventing their premature interaction. Paraffin melts when the reaction mixture reaches temperatures above 55 °C, which is sufficient to initiate specific interactions between the components of the reaction mixture. When implementing this approach, an important point is the use of mineral oil, which, when heated, ensures uniformity of paraffin melting and maintenance of homogeneity of reaction conditions (reducing the risk of aggregation of paraffin crystals and condensation of reaction mixture components).

Thus, hot-start PCR allows minimizing the probability of formation of nonspecific PCR products and the possibility of false-positive assay results.

Reverse transcription PCR (RT-PCR) is used to identify a known RNA sequence. The essence of the reaction is the synthesis of double-stranded DNA on a matrix of single-stranded RNA. For this purpose, a single-stranded RNA molecule is converted into complementary DNA (cDNA) by reverse transcription reaction (RT, reverse transcription) and then the DNA matrix is amplified using traditional PCR The use of revertase is associated with some difficulties. First of all, this enzyme is thermolabile and therefore can be used at temperatures not exceeding 42 °C. Since RNA molecules easily form secondary structures at this temperature, the efficiency of the reaction is markedly reduced and according to various estimates is approximately equal to 5%. This disadvantage can be eliminated by using thermostable polymerase as a reverse transcriptase, which is active in the presence of Mn2+ ions. It is the only known enzyme capable of both polymerase and transcriptase activityOT-PCR is widely used to detect viruses whose genome is represented by RNA (HIV, hepatitis C, influenza viruses, and others), to diagnose genetic diseases, and to semi-quantify specific RNA molecules in a cell or tissue as an indicator of the expression of the corresponding genes [1,5,6].

Multiplex (multiprimer) PCR is the simultaneous amplification of two or more desired DNA sequences in a single tube. Each pair of primers for multiplex PCR should have strict specificity with respect to the corresponding desired target, and the cycling conditions should provide equally efficient annealing of all primer pairs involved in the reaction, so that the yield of amplified products was as equal as possible. The advantage of this method is the possibility to perform screening tests with minimal expenditure on consumables. In addition, maximum information can be obtained from a single sample in a single PCR run. Nevertheless, multiplex PCR significantly limits the possibilities in the identification of low-copy samples: if a set of reagents allows the detection of four desired targets + internal control and all four targets are present in the tested sample, then in the case of a predominant amount of DNA of one or two targets, the bulk of PC components will be spent on them (competition for PC components). In case of questionable multiplex PCR results, it is recommended that the sample be analyzed using reagent kits designed to detect one specific target. It is important that the multiplex and monoplex reagent kits used to verify questionable results are from the same manufacturer [6,8,9].

In addition, other PCR variants are also possible and are most commonly used in research laboratories, such as:

Licensed under a Creative Commons Attribution 4.0 International License.

Volume 2, Issue 10, October 2024 ISSN (E): 2938-3765

Nested PCR- is used to reduce the number of reaction by-products. Two pairs of primers are used and two sequential reactions are performed. The second pair of primers amplifies a section of DNA within the product of the first reaction.

Inverted PCR is used when only a small region within the desired sequence is known. This method is useful when neighboring sequences need to be identified after DNA insertion into the genome. To do this, a series of DNA cuts are made with restriction enzymes and then the fragments are joined together.
Asymmetric PCR is performed when it is necessary to amplify predominantly one of the strands of the original DNA. It is used in some sequencing and hybridization analysis techniques. The PCR itself is performed as usual, except that one of the primers is taken in large excess.

Molecular colony method. This modification is based on the use of acrylamide gel, which is polymerized with all its components on the surface prior to PCR. During the reaction, amplification with the formation of molecular colonies occurs at the points containing the analyzed DNA.

Long-range PCR – PCR variant for amplification of long DNA stretches (10 thousand or more bases). To implement this approach, a mixture of two polymerases is used: Taq-polymerase, which is characterized by high processivity and is able to synthesize a long DNA strand in one pass; Pfu-polymerase, which has 3'→5'- exonuclease activity and is necessary to remove incomplementary nucleotides.

Group-specific PCR – PCR using conservative primers to DNA sequences for related groups within the same or between different species. The method is based on the selection of universal primers, for example, to the 18S and 26S ribosomal genes to amplify a species-specific intergenic spacer.

PCR with rapid amplification of cDNA ends (RACE-PCR). It is important to keep in mind that the PCR method for clinical diagnostic purposes is classified as a direct diagnostic method, i.e. it is aimed at direct detection of the pathogen (its genetic material) in the sample. Direct methods also include bacterioscopic, virologic, bacteriologic, and immunofluorescent methods. In contrast, indirect (serologic) methods are aimed at detecting the body's response to pathologic exposure and/or therapy (most commonly enzyme-linked immunosorbent assay (ELISA) [7,9,10,11].
In this regard, the PCR method in most of its modifications (if DNA is used as the target) does not allow assessing the effectiveness of the therapy. This is due to the fact that DNA is a rather stable product, is slowly destroyed by DNAases and persists in the body for a long time (for several weeks in case of a pronounced infectious process), even when antibacterial therapy (or antiviral therapy, if the virus genome is represented by DNA) has been effective. To assess the effectiveness of therapy at the stage of its implementation or immediately after its completion, a modification of the PCR method was developed - NASBA technology (Nucleic acid sequence-based amplification). The technique is based on amplification of RNA molecules of pathogens with the

Licensed under a Creative Commons Attribution 4.0 International License.

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

participation of enzymes: reverse transcriptase, RNA polymerase and RNase, and two pairs of specific primers [4,5,9].

Thus, the choice of RNA as the desired target is due to the fact that this nucleic acid is secreted only from living cells and is rapidly degraded after cell death. With the participation of enzymes, RNA+cDNA complexes are sequentially formed, where each element equally serves as a matrix for accumulation of RNA amplicons. The NASBA technology differs from OT-PCR in that the RNA sought during amplification is not only the basis for cDNA production, but also the main object of accumulation and detection.

References

1. Kudratova Z. E. et al. Current modern etiology of anemia //Open Access Repository. – 2023. – T. 10. – №. 10. – C. 1-4.

2. Burxanova D. S., Umarova T. A., Kudratova Z. E. Acute myocarditis linked to the administration of the COVID 19 vaccine //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 11. – С. 23-26.

3. Кудратова З. Э. и др. Атипик микрофлора этиологияли ўткир обструктив бронхитларининг ў зига хос клиник кечиши //Research Focus. - 2022. - Т. 1. - №. 4. - С. 23-32.

4. Kudratova Z. E, Normurodov S. Etiological structure of acute obstructive bronchitis in children at the present stage - Thematics Journal of Microbiology, 2023. P.3-12.

5. Kudratova Z. E., Tuychiyeva S. K. Atipik mikroflora etiologiyali o'tkir obstruktiv bronxitlar etiopatogenezining zamonaviy jixatlari. Research Focus, 2023, B. 589-593.

6. Kudratova Z. E., Karimova L. A. Age-related features of the respiratory system. Research Focus,Том 2, P. 586-588.

7. Исомадинова Л. К., Даминов Ф. А. Современная лабораторная диагностика хронического пиелонефрита у детей //Journal of new century innovations. – 2024. – Т. 49. – №. 2. – С. 112-116.

8. Isomadinova L. K., Daminov F. A. Glomerulonefrit kasalligida sitokinlar ahamiyati //Journal of new century innovations. – 2024. – Т. 49. – №. 2. – С. 117-120.

9. Isomadinova L. K., Qudratova Z. E., Shamsiddinova D. K. Samarqand viloyatida urotiliaz kasalligi klinik-kechishining o'ziga xos xususiyatlari //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 10. – С. 51-53.

10. Isomadinova L. K., Qudratova Z. E., Sh B. F. Virusli gepatit b fonida Covid-19 ning klinik laborator kechish xususiyatlari //Journal of new century innovations. – 2023. – Т. 30. – №. 3. – С. 60-65.

11. Isomadinova L. K., Yulayeva I. A. Buyraklar kasalliklarning zamonaviy diagnostikasi //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 10 Part 3. – С. 36-39

12. Kudratova Zebo Erkinovna, Tamila Abdufattoevna Umarova, & Sirojeddiova Sanobar. (2024). Modern types of immunoenzyme analysis methods old problems. Web of Discoveries: Journal of Analysis and Inventions, 2(6), 67–70.

Licensed under a Creative Commons Attribution 4.0 International License.