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METHODS OF AMPLIFICATION OF DNA FRAGMENTS

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Much of the application of genetic engineering technologies depends on the ability to obtain a large number of copies of a particular DNA fragment. The PCR method is a key technology that has enabled a revolution in biotechnology - it has become possible to obtain many thousands of copies of a small DNA sample and then analyse it [1,2,3].

Keywords: sample, thermocycler, biotechnology, technology, DNA sections, amplifier, cooling, heating.

Amplification is carried out in instruments called either amplifiers or PCR Machine or PCR Thermocycler [20,21,22].

When the instrument is designed to record amplification products in real time, the definition 'real-time' (PCR-RT or PCR-Real Time) is added to the name [23].

The amplifier provides the mode necessary for PCR: periodic cooling and heating of tubes, usually with an accuracy of at least 0.1°C. The instrument performs a certain number of alternate heating and cooling cycles (thermocycles) depending on the method used and repeats them many times, adding DNA primer at the end. The copying of DNA strands occurs thousands of times [1,2,3,4].





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For best results, the temperature regimes should be changed within a minimum amount of time. In the amplifier, the cycle temperature can be reached in a few seconds, even starting from remote values of the last set point. The necessary changes take place while maintaining perfect homogeneity between the different points of the unit. In addition, the system can be programmed to create conditions of linearly and gradiently varying temperature across the width of the block [5,6,7,8]. In this way, the points with the highest level of performance can be identified and optimised. The most popular amplifiers are compact, reliable in operation and affordable amplifiers from American and German companies [9,11,12].

The analytical process of PCR determination consists of the following steps: Sample preparation \rightarrow DNA isolation \rightarrow Preparation of reaction mixture \rightarrow PCR \rightarrow PCR \rightarrow Processing of results. PCR results are processed in different ways depending on the final goal of the experiment According to the classical method, PCR products are electrophoresed in agarose gel and electrophoregrams are analysed to identify components of interest to the researcher. When real-time PCR products are recorded, software is used to process the results and analyse them. In cases where amplification products are supposed to be analysed using a genetic analyser, they are subjected to the Sanger sequencing reaction, during which each nucleotide is tagged with a special fluorescent tag, and then the samples are placed in a genetic analyser - a device that allows to determine the sequence of nucleotides in the studied section of DNA [10,14,15,16].

The real-time PCR method is also widely used for practical purposes, for example, it is the main method for the quantitative determination of GMOs and GMIs in food raw materials and food products [13].

Molecular genomics is used to solve various problems of medicine and medical genetics. For example, information and advisory genetic centres have been established in a number of countries around the world, and in France the computer expert system SESAM (Systeme Expert Specialisee aux Analyses Medicales) has been developed and is used in practice to determine a person's propensity to various diseases. It includes an expert system of disease risk assessment based on numerous laboratory (immunological, biochemical, serological and genetic) tests (more than 80), a programme for training doctors in the basics of molecular medicine, medical





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counselling based on the results of laboratory tests and a popular guide for the public [15,16,17,18].

The new generation of DNA sequence decoding technologies, which allows reading genetic texts with unprecedented speed and productivity, has not only found wide application in biomedical research, but has also become a prerequisite for impressive scientific achievements [19,20,21].

The successes of modern biology are largely determined by the rapid progress of biological instrumentation. Automation of routine procedures, miniaturisation, combining various modules into integrated multifunctional systems - all this has led to a rapid increase in the productivity of individual biological experiments and, in general, to raising research to a qualitatively new level. The active use of design solutions from other areas of technology has greatly facilitated and accelerated this process. The most striking example of a breakthrough in biology that cannot be realised without appropriate technological support is the decoding of genomes of organisms of different taxonomic groups [22,23].

The Wellcome Trust Sanger Institute (Wellcome Trust Sanger Institute, Hinxton, UK), where much of the human genome has been decoded, has a laboratory the size of a basketball gym, packed with automated sequencers running continuously in a 384-well microtiter plate format. Automation of the sequencing process has ensured that 3253037807 base pairs of human DNA have been read [21,24,25].

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