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MODERN METHODS OF GENETIC ENGINEERING

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Genetic engineering is the in vitro construction of functionally active genetic structures - recombinant DNA, i.e. artificially created genetic programmes. It is a system of experimental techniques that allow to construct in vitro artificial genetic structures in the form of recombinant (hybrid) DNA molecules and transfer the created gene constructs into a living organism, resulting in their incorporation into the genome and active functioning in the organism and its offspring [4,5,6].

Keywords*:* recombinant DNA, genetic engineering, gene, hybrid, experiment.

Recombinant DNAs become an integral part of the recipient organism and give it new unique genetic, biochemical and physiological properties. The possibility of direct (horizontal) transfer of genetic information from one biological species to another was proved by F. Griffith's experiments with pneumococci (1928). However, genetic engineering as a recDNA technology began its history in 1972, when the first recombinant DNA (recDNA) was obtained in the laboratory of P. Berg (Stanford University, USA), in which DNA fragments of lambda phage and Escherichia coli were combined with the circular DNA of monkey virus SV40 [1,2,3].

Genetic engineering achievements were most actively introduced into practice in the 80s of the 20th century, and since 1996 genetically modified plants have been used in agriculture, the commercial production of which has increased almost a hundred times by now. Genetic engineering has also helped to create lines of animals resistant to viral diseases and breeds of animals with useful traits for humans. For example, microinjection of recombinant DNA containing the bovine somatotropin gene into a rabbit zygote allowed to obtain a transgenic animal with hyperproduction of this hormone. The obtained animals had pronounced acromegaly [4,5,6,7,8].

Thus, the task of genetic engineering is genetic modification of organisms, which is mainly aimed at:

(a) making plants resistant to pesticides (e.g. certain herbicides);

(b) making plants resistant to pests and diseases (e.g. Bt modification);

(c) enhancing animal performance (e.g. rapid growth of transgenic salmon); (d) giving organisms other special qualities [9,10,11,12].

Genetic engineering methods are based on obtaining fragments of the original DNA and their modification.

Several methods are used to obtain the original DNA fragments from different organisms:

- obtaining DNA fragments from natural material by cutting the original DNA using specific nucleases (restrictionases); specific cleavage of DNA by restriction nucleases, which accelerates the isolation and manipulation of individual genes;
- direct chemical synthesis of DNA, e.g., to create probes;
- synthesis of complementary DNA (cDNA) on mRNA matrix using reverse transcriptase enzyme (revertase) [13,14,15].

Sequencing of the purified DNA fragment makes it possible to determine the nucleotide order and sequence of coded amino acids, as well as the boundaries of the gene under study. Determination of the nucleotide composition of DNA fragments according to the classical technique was previously performed using radioactive probes - DNA molecules with a previously known structure, which include radioactive isotopes of phosphorus or hydrogen. If the structure of the isolated fragment is at least partially complementary to the structure of the probe,

DNA-DNA hybridisation takes place, and the microphotograph of the preparation shows light from the radioactive isotope [16,17,18].

At present, fluorescent tags are widely used for the determination of DNA nucleotide sequences as safe for human health. An important stage of genetic engineering research is the construction of recombinant DNA, based on the binding of complementary sequences of single-stranded fragments and the production of double-stranded hybrid DNA. This recombinant DNA is then cloned by in vitro amplification using polymerase chain reaction (PCR) or by introducing a DNA fragment into a bacterial cell, which then replicates the fragment in millions of copies. Once the recombinant DNA has been isolated, it is introduced into cells or organisms using DNA transfer vectors [19,20,21,22].

The vector includes at least three groups of genes:

1. Target genes of interest to the experimenter.
2. Genes responsible for replication of the vector, its integration into the host cell DNA and expression of the desired genes.
3. Marker genes (selective, reporter genes), the expression of which can be used to judge the success of transformation (e.g., antibiotic resistance genes or genes responsible for the synthesis of proteins that glow in ultraviolet light) [23,24,25].

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