In medicine, genetic engineering is used to solve two main tasks. 1) Obtaining drugs for the treatment and prevention of diseases. Cells of bacteria, fungi, and mammals are used. 2) Gene therapy is the introduction of a normal copy of a gene into human cells. Genetic engineering is a large complex branch of genetics, which includes various issues of molecular biology, work with cell cultures, isolation and purification of biologically active substances, and so on. The presented material will present only the main points of working with recombinant DNA. Students should understand the stages of carrying out genetic engineering manipulations, the specifics of working with prokaryotic and eukaryotic cells, and the achievements of genetic engineering in the field of medicine.

First, let's look at a few definitions.

Genetic engineering is a modern field of biotechnological research aimed at obtaining organisms with new specified properties through recombination of DNA molecules.

Genetic engineering is a set of techniques, methods, and technologies for obtaining recombinant RNA and DNA, isolating genes from an organ, manipulating genes, and injecting them into other organisms. The purpose of these techniques is to achieve a change in the hereditary, genetic apparatus of the cell.

Genetic engineering is the artificial construction of functionally active genetic structures and genetically modified organisms.

Genetic engineering is the targeted modification of bio-objects as a result of the introduction of artificial genetic programs.

Genetic engineering is a set of procedures that allow the transfer of genetic material from one organism to another.

Genetic engineering is a set of methods that make it possible to create an artificial genetic structure, introduce this construct into the body and ensure its functional activity.

The main stages of genetic engineering work

1) Obtaining a gene encoding the necessary trait.

2) The creation of an artificial genetic construct is the inclusion of a gene in a vector for transfer to a recipient cell.

3) Transfer of a vector containing the desired gene and regulatory elements into a mutable cell or organism.

4) Identification and selection of cells that have received the necessary gene and which have shown the necessary trait.

Two types of enzymes are widely used in genetic engineering - restrictases and ligases. Restrictases belong to the group of endonucleases, that is, they are able to cut a DNA molecule in the middle. Restrictases recognize a certain sequence of nucleotides (or a restriction site), activate and destroy phosphodiester bonds between nucleotides in a certain section of the chain. Today, about 600 different restrictases are used. DNA ligases are capable of forming phosphodiester bonds between nucleotides. These enzymes combine (often referred to as crosslinking) DNA fragments into a single molecule.

Methods of obtaining genes

1. Isolation of a gene from DNA. The isolated DNA is subjected to cleavage by restrictases, followed by separation of the desired fragment. To do this, it is necessary to know the structure of the desired DNA region and determine the presence of restriction sites. Possible problems are related to the difficulty of selecting a re-strictase to isolate exactly the desired DNA fragment. The resulting fragments often contain "extra" sequences of nucleotides. Native DNA molecules may contain several restriction sites for the used enzymes, therefore, several DNA fragments may be formed. It will be necessary to isolate the necessary gene from the fragments that have been swept away.

2. Gene copying by PCR. Conveniently and quickly, it is possible to copy a strictly defined sequence. To do this, it is necessary to know the structure of the gene and select primers for the DNA regions that are located on the border of the desired gene.

3. Synthesis of a gene based on isolated messenger RNA. The enzyme RNA-dependent DNA polymerase (reverse transcriptase) is used for this purpose. A single strand of DNA nucleotides is synthesized on the basis of mRNA. Then, DNA-dependent DNA polymerase synthesizes a second DNA strand based on the principle of complementarity. It is known that mature mRNA contains only exons after the end of splicing. Therefore, the resulting gene does not contain introns. This situation is convenient when working with bacteria,

since the genes of prokaryotes are devoid of introns and splicing does not occur in them.

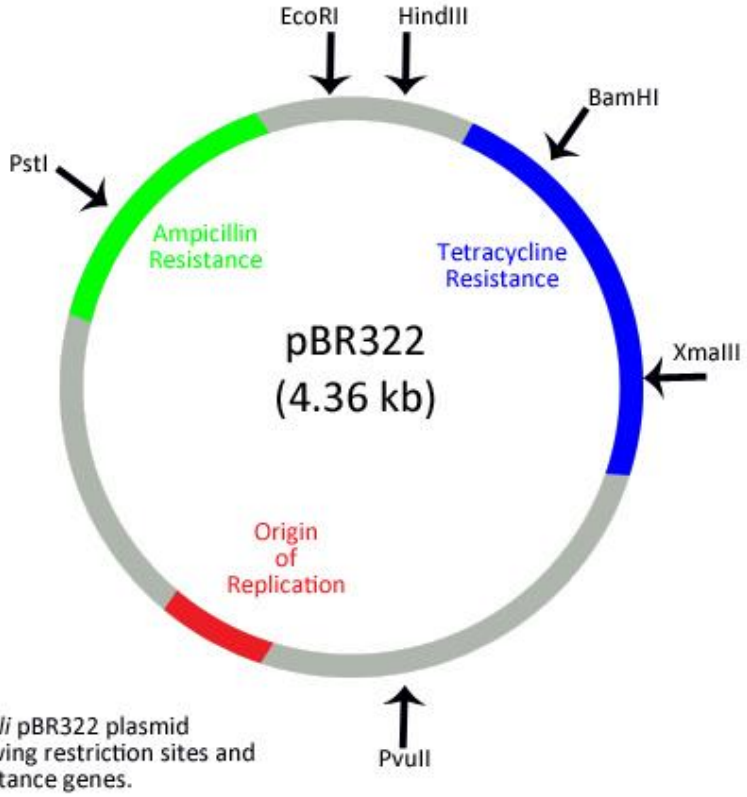
4. De novo gene synthesis ("from scratch") using chemical and enzymatic methods. To do this, it is necessary to know the structure of the necessary gene. Another option is also possible. We determine the structure of the target protein. Taking into account the genetic code, we create a sequence of DNA nucleotides. Then we synthesize the necessary gene. In this case, the synthesized DNA fragment will also contain only exons.

It must be remembered that the presence of a target gene is not enough to solve all problems. We need to get a functioning genetic construct, that is, we need to transcribe the gene and synthesize the corresponding protein. Thus, it is necessary to have regulatory elements. It is necessary to include a promoter for the gene and a terminator in the created genetic construct. It is possible to include other regulatory elements (enhancers, silencers, and others). The nature of further actions depends on the nature of the recipient cell.

Working with bacteria

Bacteria are widely used to produce proteins used for medical purposes.

2) The creation of an artificial genetic construct is the inclusion of a gene in a vector for transfer to a recipient cell. A vector is an artificial construct that is used to transfer genetic material into a recipient cell and, as a rule, is capable of self-replication. Plasmids are often used as a vector for bacteria. Plasmids are small ring-shaped DNA molecules located in the cytoplasm of a cell separately from the nucleoid. The plasmid is capable of transferring a small insert (up to 10,000 pairs of nucleotides) into the cell. Currently, thousands of plasmids have been synthesized that can be used in genetic engineering. Marker genes are often introduced into the plasmid, which make it possible to detect the cells containing this plasmid.

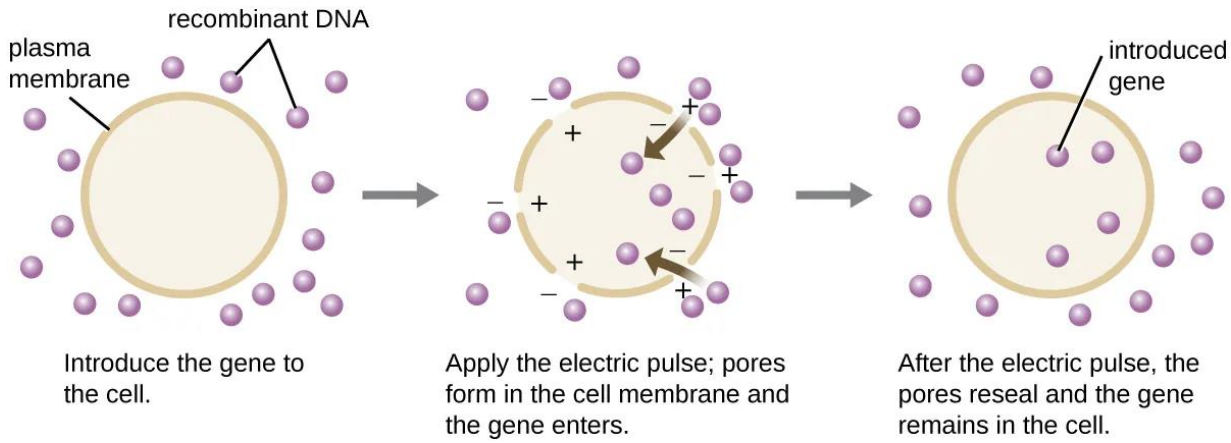


E. coli pBR322 plasmid showing restriction sites and resistance genes.

The figure shows the structure of the artificial plasmid pBR322. The plasmid contains 4,362 pairs of nucleotides. It involves the beginning of ori (Origin of replication) replication. Therefore, the plasmid can provide DNA synthesis and reproduce in E. Coli. The plasmid contains resistance genes to two antibiotics— ampicillin and tetracycline. This can be used to detect cells containing this plasmid. There are more than 40 restriction sites for various restrictases in the plasmid (some of them are marked with arrows in the figure). This makes it possible to cut the plasmid and introduce artificial genetic constructs into it.

3) Transfer of a vector containing the desired gene and regulatory elements into a mutable cell or organism. Let's name two main methods that make it possible to introduce a created plasmid into a cell.

A) Bacteria are treated with $CaCl_2$, plasmids are added and briefly heated to 42^0C (subjected to heat shock). This effect causes a phase transition of membrane lipids from a bilayer state to an unusual hexagonal state. This phase transition is accompanied by rapid absorption of exogenous DNA molecules (including plasmids created by us).

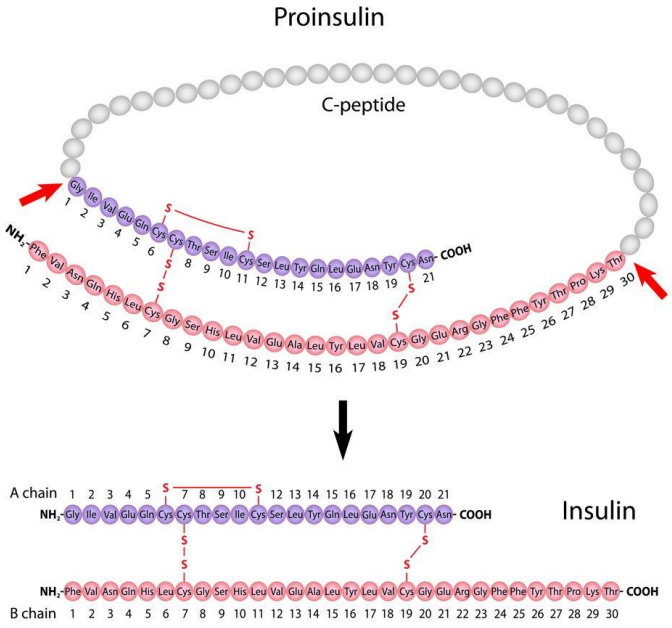


The bacteria are placed in a medium containing plasmids. Short-term (10-20 milliseconds) current is applied with a voltage of 200-300 volts. Pores are created in the cell membrane. Under the influence of osmosis (along the concentration gradient), plasmids enter the cytoplasm of the cell. The cell swells at the same time. After the termination of the electromagnetic field, the membrane integrity is restored and the plasmid remains in the cell. Some of the cells die. From 60% to 80% of the surviving cells contain plasmids. Please note that the electroporation method is universal. It allows you to work with both prokaryotic and eukaryotic cells.

4) Identification and selection of cells that have received the necessary gene and which have shown the necessary trait. As a rule, the selection process is carried out in 2 stages. First, we determine the presence of plasmids in the cells. As an example, let's consider working with the plasmid pBR322. The plasmid contains genes encoding resistance to ampicillin and tetracycline. The modified cells are grown on a nutrient medium containing one of these antibiotics. Antibiotic-resistant bacteria multiply and form colonies. These bacteria contain a plasmid. In the future, the presence and activity of the gene we need are determined. PCR can be used. DNA is isolated from bacteria and primers are selected that are complementary to the boundaries of the target gene (or to a fragment of the gene). A positive result indicates the presence of the desired gene in the cells. Next, the bacteria are cultured and the presence of the protein we need in the medium is determined. The most productive strains are

selected and used in the production of proteins. Protein production is a very complex process. The product must be isolated from the nutrient medium, purified from impurities, brought to the desired concentration, and so on. One more problem needs to be mentioned. In eukaryotic cells, almost all proteins undergo posttranslational modifications. This has to be taken into account when producing the right substances.

Consider insulin as an example. The cell produces a large protein, proinsulin, from which a special enzyme separates a fragment called a C-peptide. As a result of this treatment, insulin and C-peptide are formed.



B When the first variants of genetically engineered insulin were obtained, different strains of E. coli produced A and chains of insulin. Proteins were purified, incubated under certain conditions, disulfide bonds were formed, and an insulin molecule appeared. Currently, bacteria produce proinsulin, the protein is processed with enzymes and insulin is formed.

Working with human cells.

1) Obtaining a gene encoding the necessary trait. This stage is described above.

2) The creation of an artificial genetic construct is the inclusion of a gene in a vector for transfer to a recipient cell. Viruses are most often used as a vector. The virus interacts with the target cell using receptors. The interaction of the receptors determines the tropicity of the virus (the ability to penetrate into the cells of certain tissues). The selectivity of the virus is not absolute. Tropicity

makes it possible to limit the types of cells with which the virus interacts. After entering the cell, the virus loses its capsid and the nucleic acid of the virus penetrates into the nucleus. To date, a number of viral vectors have been proposed for effective transduction of genetic material at the organizational level, each with its own advantages and disadvantages. Vectors based on adenoviruses, adeno-associated viruses, herpes simplex virus, retroviruses and lentiviruses have received the greatest development. They differ in the nature of the target cells, the size of the transferred genetic construct, and the intensity of the immune response to the virus. It is necessary to isolate adeno-associated viruses (AAV). They cannot reproduce unless there is another virus in the target cell (for example, an adenovirus). These viruses are not pathogenic to humans. They contain single-stranded DNA, which makes it easier to work with genetic material. AAVs have several serological variants, which differ in antigens and tropicity to various human cells. This feature allows you to select a variant of the virus for action on specified target cells. Upon entering the cell, many AAVs do not integrate into the chromosome, but form an independent circular DNA molecule in the nucleus. This structure is called an episome. This feature reduces the risk of negative effects of gene therapy. Episomes persist for a long time in non-dividing cells and ensure the expression of the implanted gene. In 2026, both genetically engineered drugs registered in Russia (zolgensma and luxturna) were created on the basis of AAV. When creating a vector, the genetic material of the virus is partially or completely removed. The desired gene is inserted into the vector. In this case, constitutive promoters are often used. Usually, transcription factors are needed for the interaction of the promoter with RNA polymerase and the activation of the enzyme. The constitutive promoter independently ensures the activation of RNA polymerase. This feature makes it possible to obtain a high level of expression of the embedded genetic material.

3) Transfer of a vector containing the desired gene and regulatory elements into a mutable cell or organism. The resulting vector is injected into the person. The method of administration depends on the nature of the target cells.

Advances in genetic engineering (drugs that are used in healthcare

practice)

The drug Luxturna is a gene therapy drug. It is used to treat adults and children with vision loss as a result of hereditary retinal dystrophy caused by mutations in the RPE65 gene. These mutations prevent the body from producing a protein necessary for vision, leading to decreased vision and possible blindness. The active substance of the drug is a modified AAV containing a working copy of the RPE65 gene, which, after injection, delivers this gene to retinal cells. This gives the retina the ability to produce proteins necessary for vision. The virus used for gene delivery does not cause human diseases. The CAG promoter was used. It is a synthetic hybrid promoter that is used for high and widespread expression of transgenes in mammalian cells. The name "CAG" is given by the name of three main components: the early cytomegalovirus enhancer (CMV), the chicken β-actin promoter (CBA) and the rabbit β-globin gene splicing acceptor. The drug Luxturna is prescribed only if the results of a genetic study show that vision loss is caused by mutations in the RPE65 gene.

Zolgensma is a gene therapy drug designed to introduce a functional copy of the motor neuron survival gene 1 (SMN1) into transduced cells to eliminate the monogenetic root cause of spinal muscular atrophy (SMA). It is expected that by introducing an alternative source of SMN protein expression into motor neurons, the drug will ensure the survival and correct functioning of the transduced motor neurons. Zolgensma is a non-replicating recombinant adeno-associated viral vector that uses the capsid of the adeno-associated serotype 9 virus (AAV9) to deliver a stable, fully functional human SMN transgene. It has been established that capsid AAV9 is able to penetrate the blood-brain barrier and transduce motor neurons. The SMN1 gene contained in the preparation is designed to persist in the nucleus of the transduced cells in the form of episomal DNA. It is assumed that it will be stably expressed for a long time in postmitotic cells. The ability of the AAV9 virus to cause disease in humans is unknown. The transgene is introduced into target cells as a self-complementary double-stranded molecule. Transgene expression is regulated by a constitutive promoter (a hybrid of the chicken P-actin gene promoter and cytomegalovirus transcription enhancer), which ensures constant and stable

expression of the SMN protein.

Insulin

Human follicle-stimulating hormone (Primapur drug)

Recombinant human albumin. The active pharmaceutical substance is made from a recombinant engineered strain of Pichia Pastoris yeast with high expression of human albumin and obtained as a result of fermentation, preparative isolation, coarse and fine purification, including affinity chromatography, ultrafiltration, and concentration. These three drugs are produced in the Sverdlovsk region on the basis of "Medsintez".

Growth Hormone

Interferon-alpha

Interferon-gamma

Erythropoietin

Human granulocyte colony stimulating factor

Human recombinant Blood Coagulation Factor VIII

The hepatitis B vaccine. It is a preparation based on the surface antigen of the hepatitis B virus, obtained by DNA recombination in yeast culture, transformed by including in their genome a gene encoding the surface antigen of the hepatitis B virus.

The Gardasil vaccine. Quadrivalent vaccine against human papilloma virus (HPV). The vaccine is a mixture of highly purified virus-like particles of the recombinant basic capsid protein (L1) of HPV types 6, 11, 16 and 18. L1 proteins are produced by separate fermentation in recombinant yeast cells Saccharomyces cerevisiae and form virus-like particles by self-assembly. Virus-like particles for each type are purified and adsorbed onto an aluminum-containing adjuvant (amorphous aluminum hydroxyphosphate sulfate). It is used for the prevention of diseases caused by human papillomavirus types 6, 11, 16 and 18. These include cancer of the cervix, vulva and vagina; genital warts.

Omalizumab, Genolar. These are recombinant monoclonal IgG antibodies that selectively bind to human DNA. They are obtained using recombinant DNA technology in a system for expression provided by Chinese hamster ovarian cells. It is used in the treatment of bronchial asthma.

Infliximab, etanercept, tocilizumab -for the treatment of juvenile idiopathic

arthritis, rheumatoid arthritis.

Tocilizumab is a recombinant monoclonal antibody against the human interleukin-6 (IL-6) receptor from the IgG subclass. Antibodies are produced using mammalian cells (Chinese hamster ovary cells). Tocilizumab binds to IL-6 receptors and inhibits IL-6-mediated signaling through these receptors.

Etanercept. It is a hybrid dimeric protein molecule consisting of a tumor necrosis factor (TNF) receptor coupled to the Fc fragment of human IgG1. It is produced using recombinant DNA technology on Chinese hamster ovarian cells. Etanercept is a competitive inhibitor of TNF binding to its receptors on the cell surface, and thus inhibits the biological activity of TNF.

Infliximab. Chimeric mouse-human IgG monoclonal antibodies consisting of a variable (Fv) region of murine monoclonal antibodies to tumor necrosis factor alpha and a fragment of the human IgG molecule. It binds to the tumor necrosis factor alpha and reduces its functional activity.

Rictusimab. Genetically engineered chimeric mouse-human monoclonal IgG antibody to CD20 antigen. The CD20 antigen is expressed on the surface of B lymphocytes. When bound to CD20, rituximab causes B cell lysis. Antitumor agent.

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