Biochemistry of genetic engineering

Genetic engineering is a scientific field dealing with the artificial recombination of synthetic and natural nucleic acids in vitro with the intention of creating new biological systems beneficial to humans.

The basic methods were developed thanks to revolutionary discoveries in nucleic acid enzymology. These methods can be used to change the genetic make-up of an organism or modify the arrangement of the genome. Editing of DNA is carried out by many methods. We will recall only the most important ones.

DNA cleavage

Defined DNA cleavage was made possible by the discovery of restriction endonucleases (restrictases). These enzymes cleave dsDNA into very specific centrally symmetric sequences of nucleotides called palindromes (see table). Restrictases are natural enzymes of bacteria, where they are part of the so-called restriction-modification system. The same sequence is methylated in the own DNA by this system, which prevents restrictases from degrading the own DNA. The system is therefore aimed at the degradation of foreign DNA whose target sequences are not protected. If the phage DNA enters the bacterium, it is mostly degraded - the phage undergoes restriction. Only exceptionally is some of the phage DNA methylated by the system, the phage survives, has been modified and can reproduce in the given bacterial strain.

The specificity of restrictases has been used in DNA enzymology. Around a hundred such enzymes have been described, they are called by abbreviations of the names of the bacteria in which they were discovered (EcoRI – E. coli, HaeIII – Haemophilus influenzae). Each restrictase has a specific target sequence in which it cleaves either opposing phosphodiester bonds of dsDNA or bonds symmetrically several nucleotides away from the center of symmetry (see table). In the second case, cohesive ("sticky") ends of the resulting restriction fragments are formed, which can reassociate, even with fragments obtained by cleavage of other DNA with the same restrictase. This option is very advantageous when recombining fragments and constructing new genes and genomes. Today, however, it is not a problem to artificially create any cohesive ends on any dsDNA fragment.

Another biotechnologically important enzyme is DNA-ligase, discovered during the study of natural DNA replication. It covalently links the 3'-OH end of the chain to the 5'-P end of the DNA.

Electrophoresis of nucleic acids

Sections of DNA, PCR reaction products or DNA grafts can basically be separated by chromatography or electrophoresis. Electrophoretic separation is much more widely used. It is usually performed in a gel, either agarose or polyacrylamide. In both cases, molecules that carry a negative charge in an alkaline environment move in an electric field from the cathode to the anode. Gels form a relatively dense network, through which larger molecules pass more slowly than smaller molecules - that is why we speak of the molecular sieve technique.

Agarose gel electrophoresis

Agarose is a polysaccharide consisting of D-galactose and anhydro-L-galactose, which is produced by some seaweeds and under the name agar is used to produce gels in the food industry, microbiology, immunology or biochemistry. Gels containing 0.5 to 4% agarose are used for nucleic acid electrophoresis. The higher the polysaccharide content, the better the distinguishing ability of the gel, but this also means that the electrophoresis process is slower and the preparation of the gel is more technically demanding.

Ethidium bromide is usually added directly to the agarose gel, so that after electrophoresis the individual fractions can be visualized by UV radiation. Another option for detection is blotting on a membrane and subsequent staining of nucleic acids or hybridization with labeled probes.

Polyacrylamide gel electrophoresis

Another carrier used in nucleic acid electrophoresis is polyacrylamide gel. Polymerization of acrylamide produces linear polyacrylamide molecules. These are connected by cross bridges, which are formed by copolymerization with N,N'-methylenebisacrylamide. Both acrylamide and methylenebisacrylamide are relatively stable substances; the polymerization takes place in the absence of atmospheric oxygen (removed by deaeration using a vacuum) and begins by mixing the catalysts ammonium peroxydisulfate (known under the older name ammonium persulfate, APS for short) and N,N,N',N'-tetramethylethylenediamine (TEMED).

APS in aqueous solution with TEMED releases free oxygen radicals that attack acrylamide and bisacrylamide molecules and thus trigger their polymerization.

The molecular sieve of the polyacrylamide gel is quite dense, so it is suitable for the separation of shorter fragments.

Because polyacrylamide is less reactive than agarose, DNA fragments can be stained by other techniques in addition to the methods used in the agarose gel. Among the classical methods, silvering is among the most sensitive, with which the amount of DNA can be detected even several orders of magnitude lower than with

ethidium bromide.

Hybridization

It is often used to identify the searched string, or its part. The searched sequence of nucleotides hybridizes with a previously prepared and labeled probe (a DNA or RNA chain, labeled with a fluorochrome or a detectable radioisotope).

Hybridization is a process in which two complementary strands join together, it is possible to combine both DNA and RNA. it can be simply divided into:

1. **denaturation of the sample** – breakdown of hydrogen bridges between bases and separation of chains (heating),
2. **adding a probe** – a nucleic acid chain whose complementary part we are looking for,
3. **renaturation** – by slow renaturation (cooling), during which complementary chains are joined,
4. **evaluation of the experiment, examination.**

Syntéza umělé DNA

The required DNA strand can be prepared in various ways. There are even automated procedures for synthesizing DNA with the desired sequence from individual nucleotides.

The basic principle is to suppress the reactivity of 3'-OH, 5'-OH and possibly also -NH2 groups, sensitive to condensing agents, on nucleotides. Their protection is achieved by binding suitable organic groups, e.g. trityl, benzoyl, acetyl, etc. These groups can be easily removed, e.g. by changing the pH, which exposes the reactive group of the nucleotide for immediate reaction.

A typical example is this arrangement: the first nucleotide is attached to a column with a glass support (small particles), on which all groups except 5'-OH are protected in the described manner. The column is then washed with a solution of another, activated nucleotide. It is usually a deoxyribonucleoside 3'-phosphoamidite that binds to the 5'-OH of the fixed nucleotide. After oxidation of trivalent phosphorus to pentavalent, a phosphodiester bond and a dinucleolide are formed.

The protection at its 5'-end is removed and the column is washed with another activated nucleotide, etc. All protective groups and methyls from the phosphates are then removed by appropriate conditions, and the finished oligonucleotide is released from the column.

The description of the procedure is greatly simplified, the synthesis of the oligonucleotide takes hours. The speed of the process does not bear comparison with the speed of natural biosynthesis (e.g. in E. coli 16,000 bases per minute). However, the ability to make DNA of any primary structure is an immense advance. Synthetic DNA fragments can be enzymatically linked into longer chains of artificial DNA.

Amplification and gene expression

Simply put, this procedure requires:

- construction of a vector – a carrier of a new gene, capable of entering the host cell,
- host cell selection - differentiate cells with a changed genotype,
- multiplication of selected host cells,
- creation of conditions for effective or economically advantageous expression of the new gene in the host cell.

Currently, all these procedures are largely mastered and used for the study of human, animal and plant genomes, for the diagnosis of molecular diseases (hereditary diseases) and their therapy, and for the production of new substances, including medicines, and also for the breeding of economic plants. Many of these uses fall under the modern industry called biotechnology.

Links

Related articles

- Identifikace restrikčních fragmentů
- Pomnožení a exprese izolovaného genu v hostitelské buňce

Sources

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References

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