Nucleic acid analysis methods

Nucleic acid analysis is based on two fundamental approaches:

- DNA multiplication: in living organisms or in vitro by a chemical process (PCR)
- molecular hybridization

Application:

- gene structure analysis, mapping, function
- analysis of genetically determined diseases
- prenatal diagnosis
- detection of carriers mutated allele
- diagnosis and pathogenesis of the disease
- biosynthesis – insulin, growth hormone...
- treatment of genetic diseases - gene therapy
- fundamental discovery for development

Selective amplification of specific DNA fragments from genomic DNA or cDNA. Amplification can be carried out "in vivo" or "in vitro" using DNA polymerase

Multiplication inside host cells

Thanks to the discovery of enzymes - restriction endonucleases - it was possible to obtain **fragments of different lengths** which can be further analyzed. Propagation itself is based on the creation of recombinant DNA molecules, which are created by combining different sections of DNA molecules originating from taxonomically different species. Another important DNA molecule necessary for DNA multiplication is the so-called vector into which the DNA fragment is inserted. The recombinant vector is then introduced into a host organism, such as a bacterium.

Restriction endonucleases

Restriction endonucleases are **enzymes'' *isolated from bacteria that* *cleave double-stranded DNA'* *at specific sequences. Enzymes protect bacteria from foreign DNA. Endonucleases cleave DNA in such a way that overhanging sections of the so-called* *cohesive* *or* *sticky ends*** and DNA fragments of a defined length are formed. Mostly they are **palindromes**'.

Restriction endonucleases can cleave recombinant DNA molecules with the participation of the ``DNA-ligase *enzyme and complementarity between the sticky ends, e.g. the EcoRI enzyme isolated from E. coli cleaves the sequence GAATTC* Restrictases are needed to obtain a sufficient amount of DNA.

Vectors

- Plasmids – found in bacteria
- Lambda (λ) phage - belongs to bacteriophages
- Cosmids – a combination of plasmid DNA and lambda phage
- Artificial yeast chromosomes - possibility to clone very long fragments (up to 2Mpb)
- Bacterial artificial chromosomes

DNA Sequencing - Sanger Method

The basic method of DNA analysis is '*DNA sequencing - determining the sequence of nucleotides in DNA*.

Two methods were developed - Sanger (dideoxy chain termination method) and Maxam-Gilbert method (chemical degradation).

Sanger method

The most frequently used.

The basis is the use of a mixture of standard deoxy nucleotide triphosphates (dNTPs) and modified dideoxy nucleotide triphosphates (ddNTPs) in the preparation of the DNA segment to be sequenced. Modified ddNTPs result in termination of DNA synthesis. The result of ``DNA synthesis *is a series of DNA molecules of different lengths, each differing in length by ``one nucleotide*.

The whole process takes place in 4 separate enzyme reactions. Each contains - **template DNA** in single-stranded form, **DNA-polymerase**, **primer**, 4 **dNTPs** and a modified nucleotide ***dideoxy ``nucleotide triphosphate* (ddNTPs). In each of the 4 reactions there is a different ddNTP - ddATP, ddGTP, ddCTP, or ddTTP.**

After sequencing, the DNA molecules are *separated by capillary electrophoresis* - the products pass through the capillary, where they are separated according to size. If radioactively labeled dNTP is added to the sequencing reaction - it is possible to detect the resulting DNA on x-ray film in the form of visible bands of all 4 lines.

Multiplication of DNA in vitro - polymerase chain reaction

The polymerase chain reaction, the so-called PCR, is the repeated replication of DNA ``in vitro *with each replicated DNA molecule serving as a template: denatured DNA → primer attachment → DNA synthesis.*

🔎 *For more information see PCR.*

Nucleic acid hybridization

NK hybridization = joining according to base pairing rules.

It consists in the denaturation of the DNA double helix and subsequent renaturation. It can occur between two strands of DNA, DNA and RNA, or RNA and RNA. Based on hybridization, we can use a probe to detect a complementary nucleic acid sequence.

Probe

A DNA or RNA molecule that, based on hybridization, can be used to detect the '*complementary sequence* of a nucleic acid.

They must be used in **single-stranded form** and labeled so that the hybridization reaction can be visualized.

As probes can be used - **cloned DNA sequences**, sequences **obtained by PCR**, synthetic **oligonucleotides**, RNA obtained by transcription of *cloned DNA* in vitro.

Southern blotting

The basis is the isolation and purification of DNA, followed by cleavage with a restriction enzyme to produce thousands of fragments of different sizes. The next step is electrophoretic separation according to the size of the fragments. The gel is then immersed in an alkaline solution, which denatures the DNA. This is followed by blotting, which is the transfer of denatured fragments to a membrane that is exposed to a temperature of 80 °C and the fragments are fixed on it. Then the hybridization itself occurs.

🔎 *For more information see Southern blotting.*

Northern blotting

This is a similar technique to Southern blotting, but the analyzed acid is RNA.

Microchip Technology

A DNA microarray is usually a glass slide onto which thousands of different DNA sequences are attached in a specific order. These molecules serve as probes for hybridization with test samples. Use of microchip technology:

- Analysis of gene expression - comparison of expression in cells and tissues under different situations (tumor cells)
- Genotype analysis – determining whether an individual is homozygous or heterozygous for a given polymorphism
- Genetic testing – identification of the mutation carrier, diagnosis of a hereditary disease

Links

Related Articles

- Southern Blotting
- DNA
- PCR
- Nucleic acid denaturation, molecular hybridization

Source

- KOHOUTOVÁ, Milada. *Lékařská biologie a genetika. (II. díl).* 1. edition. Karolinum, 2012. ISBN 978-80-246-1873-9.

- ŠTEFÁNEK, Jiří. *Medicína, nemoci, studium na 1. LF UK* [online]. [cit. 11/02/2010]. <https://www.stefajir.cz/>.