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СЕКВЕНИРОВАНИЕ ДНК И ИССЛЕДОВАНИЯ В ОБЛАСТИ ГЕНОМИКИ

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Ключевые слова: секвенирование ДНК, ген, геном,геномика, хромосома.

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DNA SEQUENCING AND GENOMICS RESEARH

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The review is devoted to DNA sequencing and human genome research for the purpose of diagnostics, prevention and treatment of diseases. This review also covers the application of genetic and molecular-biological research methods in modern medicine.

Key words: DNA sequencing, gene, genome, genomics, chromosome.

The development of nucleic acid cloning and sequencing methods has significantly advanced molecular biology [2, 3, 4, 5]. Knowledge of the primary structure of the genome has made it possible to use new methods of genetic engineering, such as directed mutagenesis and in vitro recombination, to study the functions of regions of genetic material [2, 3, 4, 5 7, 8].

By manipulating nucleotide sequences, genomes with new functions can be created [1, 2, 3]. Nucleic acid sequencing has become routine in molecular biology, and even more advanced automated sequencers are expected to become available, leading to an increase in the number of sequences decoded [1, 3, 5].

Knowledge of the genetic code allows the identification of regions encoding potential proteins, which is key to understanding the functional structure of nucleotide sequences [7, 8].

DNA sequencing is the process of determining the sequence of nucleotides in an organism's genome [3, 4, 6]. It is a key technology that allows scientists to study genomes, identify genes, and understand their functions [6]. With the development of DNA sequencing, new research methods in genomics have emerged that have led to significant discoveries in biology and medicine [6].

Genomics plays an important role in understanding the mechanisms of development of organisms, their adaptation to the environment, and in identifying genetic diseases and developing treatments for them [4, 8]. In the modern world, DNA sequencing and genomics research have become an integral part of the work of many scientists and specialists in various fields of science [2, 3, 4, 5 6, 7, 8].

The aim

The aim of this study is to review the scientific literature data on DNA sequencing and human genome research for the purpose of diagnostics, prevention and treatment of diseases, as well as the application of genetic and molecular-biological research methods in modern medicine [1, 2, 3, 4, 5 6, 7, 8].

Material and methods

Currently, the most popular methods of DNA sequencing are the Edman Method, the Sanger Method and pyrosequencing [2, 3, 4, 5 6, 7, 8]**.**

The Edman method, developed by Per Victor Edman in 1950-1956, was one of the earliest sequencing methods. The essence of the method is the sequential treatment of the peptide under study with a certain set of reagents, which leads to the cleavage of one amino acid from the N-terminus of the sequence. Repeating the reaction and analysing the products provides information on the amino acid sequence of the peptide [2, 3, 4, 5].

The reagent FITC (phenylisothiocyanate) is used to determine the N-terminal amino acid in a peptide. It reacts with alpha-amino acids and the alpha-carboxyl group of free amino acids to form a phenylthiohydanthion derivative with the N-terminal amino acid of the polypeptide. This allows selective hydrolysis of the bond between the alpha-carboxyl group of the N-terminal amino acid without damaging other peptide bonds. The FITC complex with the N-terminal amino acid is isolated and identified by chromatography, thus obtaining the amino acid sequence of the peptide [2, 3, 4, 5].

The Sanger method, also known as the dideoxynucleotide method or "chain break" method, was developed by F. Sanger in 1977 and is a widely used method for determining the nucleotide sequence of DNA. In Sanger sequencing, a synthetic oligonucleotide of 17-20 strands in length is hybridised with a specific section of one of the strands to be sequenced. This oligonucleotide is a primer that initiates synthesis of the complementary chain by providing a 3'-hydroxyl group to initiate synthesis [2, 3, 4, 5, 7, 8].

The solution with primer is distributed into four tubes containing four deoxynucleotides (dATP, dCTP, dGTP and dTTP), one of which is labelled with a radioactive isotope, and one of four 2',3'-dideoxynucleotides (ddATP, ddTTP, ddGTP or ddCTP). The dideoxynucleotide is incorporated at different positions in the growing chains, causing the chain to stop growing after its addition [2, 3, 4, 5, 7, 8].

The processing results in a unique set of oligonucleotides of different lengths incorporating the primer sequence when DNA polymerase is involved. After the addition of formamide for chain divergence and four-track electrophoresis in a polyacrylamide gel, radioautography is performed to "read" the nucleotide sequence of the sequenced DNA strand [2, 3, 4, 5, 7, 8].

In a more modern approach, dideoxynucleotides are labelled with four fluorescent dyes and PCR is performed in a single tube. Then, during electrophoresis in a polyacrylamide gel, a laser beam excites the fluorescence of the dyes, which allows us to determine which nucleotide is migrating at a given moment. Modern DNA sequencing instruments use capillary electrophoresis [2, 3, 4, 5, 7, 8].

Pyrosequencing is a DNA sequencing method based on the principle of "sequencing by synthesis". When a nucleotide is incorporated, the released pyrophosphates are detected. The technology was developed by Paul Niren and his student Mustafa Ronaghi at the Royal Institute of Technology (Stockholm) in 1996 [2, 3, 4, 5, 7, 8]..

The principle of the method is quite simple and is based on (+/-)-sequencing proposed back in the 60s. When deoxynucleoside triphosphates are added sequentially to the DNA polymerase complex, their incorporation into the synthesised strand depends on the nucleotide sequence of the matrix. DNA polymerase synthesis is accompanied by the release of pyrophosphate. This pyrophosphate, in the presence of sulfurylase and adenosine phosphosulfate, is converted to ATP and triggers the oxidation of luciferin by luciferase accompanied by bioluminescence. The luminescence is recorded by a photomultiplier tube or digital camera [2, 3, 4, 5, 7, 8]..

The instrumentation required for pyrosequencing was not very complicated at first. In the original version described by Hyman, it was proposed to use a flow capillary containing several immobilised enzymes and the DNA to be analysed. Biotage AB Corporation (Sweden) offers instruments for use with 96-well plates. Reagents are introduced into the wells by the head of an inkjet printer, after which they are not removed, but cleaved by a special enzyme – apyrase [2, 3, 4, 5, 7, 8]..

An essential feature of the more sophisticated technology developed by 454 Life Sciences is the use of emulsion PCR to simultaneously prepare hundreds of thousands of DNA preparations for sequencing in parallel [2, 3, 4, 5, 7, 8]..

DNA sequencing is a powerful tool that allows scientists to study the genetic characteristics of different organisms. By determining the sequence of nucleotides in DNA, researchers can identify genetic mutations associated with various diseases and inherited conditions. This opens new horizons in medical science, enabling the development of personalised treatments and prevention of genetically determined diseases [2, 3, 4, 5, 6, 7, 8].

DNA sequencing has been used in medicine to diagnose genetic diseases, detect inherited abnormalities and develop individualised treatment approaches. Thanks to this technology, doctors can predict the risk of certain diseases in patients, which helps to realise the concept of predictive medicine and prevent possible problems in advance [2, 3, 4, 5, 6,].

Complete genome sequencing allows scientists to study evolutionary processes, investigate genetic mechanisms of organisms' development and understand the features of heredity. This

information is of great importance not only for medical purposes, but also for expanding scientific knowledge in various fields of biology and genetics [2, 3, 4, 5 6].

DNA sequencing is an integral part of modern scientific endeavour that has revolutionised the approach to studying genetic processes. Thanks to this technology, scientists can gain a deeper understanding of the molecular mechanisms of life, develop innovative methods to treat and prevent disease, and expand our knowledge of genetics and the evolution of organisms [2, 3, 4, 5, 6, 7, 8].

Conclusion

Thus, DNA sequencing and human genome research are modern methods that allow studying the genetic material of an organism with high precision. These technologies play an important role in medicine, helping in the diagnosis, prevention and treatment of various diseases [6].

DNA sequencing allows the detection of genetic mutations that may be the cause of the development of various diseases. Thanks to this, specialists can diagnose genetically determined diseases early and take measures to prevent them[2, 3, 4, 5, 6].

Studies of the human genome can reveal individual predisposition to certain diseases. This information helps people to make decisions about their lifestyle and to consult a doctor for preventive measures. [1, 2, 3, 4, 5, 6].

Analysing a patient's genome allows the most effective methods of treatment to be selected, taking into account the individual characteristics of the organism. This helps to improve the results of therapy and reduce the risk of complications [1, 2, 3, 4, 5 6, 7, 8].

Human genome research opens up new opportunities in medicine, helping to predict, prevent and treat diseases at earlier stages. Thanks to these technologies, it is possible to individualise the approach to each patient, which improves the effectiveness of treatment and the quality of life of people [1, 2, 3, 4, 5 6, 7, 8].

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