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APPLIED MOLECULAR GENETICS LABORATORY AND TECHNOLOGIES FOR ITS DEVELOPMENT

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ANNOTATION

It was known as early as the era of primitive society that some characteristics of animals and plants could be transmitted from generation to generation, and this knowledge was used by selective reproduction in livestock and farming. However, modern genetics, as a science that understands the mechanisms of breeding, began to develop only after the work of Gregory Mendel (21st century).

Key words: basic units of breeding, basic units of breeding, construction and control of cell components

INTRODUCTION

Mendel discovered that breeding is a fundamental discrete process with independent functionalities. These basic units of breeding are now called “genes”. In the cells of the organism, genes are located in the molecules of the body’s DNA, carrying the necessary information for the construction and control of cell components in itself. Although genetics plays a big role in determining the appearance and behavior of an organism, the overall result will depend not only on genes, but also on the environment that surrounds the organism. For example, a person's height is determined not only by genes, but also by the nutrients and health that he received in childhood. The main task of genetics is to reveal the development and transition of the character and characteristics of organisms to future generations by researching the structure and functions of chromosomes, genes and nucleic acids (DNA, RNA), which are considered the material basis of heredity. Research on the emergence of hereditary variability in organisms under the influence of various physical and chemical factors and its significance in the evolution of organisms is also among the tasks of genetics. Creation of fertile varieties of cultivated plants, productive breeds and strains of animals and microorganisms; development of methods for their prevention and treatment based on the study of the causes of the appearance of hereditary diseases; genetically substantiating the preservation of the gene pool, capturing factors of the ecological environment that negatively affect heredity, represent practical problems of genetic research.

Genetic engineering, also called genetic modification or genetic manipulation, is the direct manipulation of the organism's genes using biotechnology. It is a set of technologies used to modify the genetic makeup of cells, including the transfer of genes within and across species to produce improved or new organisms. New DNA is obtained by separating and copying genetic

material using either recombinant DNA methods or artificially synthesizing by DNA. Building a is usually created and used to inject DNA into a host organism. The first recombinant DNA molecule was created by Paul Berg in 1972 by combining DNA from the monkey virus SV40 with the lambda virus. In addition to adding genes, the process can be used to remove or "knock out by knockout ", genes. New DNA can be inserted randomly, or target into a specific part of the genome. An organism formed through genetic engineering is considered to be genetically modified (GM) and the result is a genetically modified organism (GMO). The first GMO was a bacterium created by Herbert Boyer and Stanley Cohen in 1973. Rudolf Yaenisch created the first GM animal when he added foreign DNA to a mouse in 1974, the first company to focus on genetic technology, was founded in 1976 and started producing human proteins. Genetically engineered human insulin was produced in 1978, and insulin-producing bacteria were commercialized in 1982. Genetically modified food has been sold since 1994 with the release of Flavr Savr tomatoes. Flavr Savr was developed for longevity, but most of the current GM crops have been modified to increase resistance to insects and herbicides. GloFish, the first GMO produced as a pet, was sold in the US in December 2003. In 2016, the meat of Red Fish was sold modified with growth hormone.

Genetic engineering has been used in many fields such as research, medicine, industrial biotechnology and agriculture. In the study, GDO is used to study the function of genes and through loss of function, increasing function, monitoring expression and expression. By knocking out genes responsible for certain conditions, it is possible to create animal models of human diseases. Genetic engineering has the potential to treat genetic disorders, along with the production of hormones, vaccines and other drugs such as gene therapy. The technique used in the manufacture of medicines can also be used industrially, such as the production of enzymes for laundry detergent, cheese and other products.

The rise in commercialized prices for genetically modified crops has brought economic benefits to farmers in many countries, but has been the source of most sources of controversy around technology. This has existed since its initial use; the first field tests were destroyed by anti-GM activists. Although there is scientific consensus that the currently available food derived from GM crops does not pose a greater risk to human health than conventional food, since GM food safety worries critics. Gene flow, exposure to non-targeted organisms, food supply management, and intellectual property rights as potential problems have also been raised. These concerns led to the development of a regulatory framework that began in 1975. It was adopted into an international treaty, the Cartagena Protocol on Biological Safety, in 2000. Individual countries have developed their own regulatory systems in relation to GMOs, with the greatest differences between the United States and Europe.

Molecular genetics is a sub-field of biology that addresses how differences in the structures or expression of DNA molecules manifests as variation among organisms. Molecular genetics often applies an "investigative approach" to determine the structure and/or function of genes in an organism's genome using genetic screens. The field of study is based on the merging of several sub-fields in biology: classical Mendelian inheritance, cellular biology, molecular biology, biochemistry, and biotechnology. Researchers search for mutations in a gene or induce mutations

in a gene to link a gene sequence to a specific phenotype. Molecular genetics is a powerful methodology for linking mutations to genetic conditions that may aid the search for treatments/cures for various genetic diseases.

For molecular genetics to develop as a discipline, several scientific discoveries were necessary. The discovery of DNA as a means to transfer the genetic code of life from one cell to another and between generations was essential for identifying the molecule responsible for heredity. Molecular genetics arose initially from studies involving genetic transformation in bacteria. In Avery, McLeod and McCarthy isolated DNA from a virulent strain of *S. pneumoniae*, and using just this DNA were able to convert a harmless strain to virulence. They called the uptake, incorporation and expression of DNA by bacteria "transformation". This finding suggested that DNA is the genetic material of bacteria. Since its discovery in genetic transformation has been found to occur in numerous bacterial species including many species that are pathogenic to humans. Bacterial transformation is often induced by conditions of stress, and the function of transformation appears to be repair of genomic damage.

MATERIALS AND METHODS

The phage group was an informal network of biologists centered on Max Delbrück that contributed substantially to molecular genetics and the origins of molecular biology during the period. The phage group took its name from bacteriophages, the bacteria-infecting viruses that the group used as experimental model organisms. Studies by molecular geneticists affiliated with this group contributed to current understanding of how gene-encoded proteins function in DNA replication, DNA repair and DNA recombination, and on how viruses are assembled from protein and nucleic acid components (molecular morphogenesis). Furthermore, the role of chain terminating codons was elucidated. One noteworthy study was performed by Sydney Brenner and collaborators using amber mutants defective in the gene encoding the major head protein of bacteriophage T4. This study demonstrated the co-linearity of the gene with its encoded polypeptide, thus providing strong evidence for the "sequence hypothesis" that the amino acid sequence of a protein is specified by the nucleotide sequence of the gene determining the protein.

Watson and Crick (in conjunction with Franklin and Wilkins) figured out the structure of DNA, a cornerstone for molecular genetics. The isolation of a restriction endonuclease in *E. coli* by Arber and Linn in opened the field of genetic engineering. Restriction enzymes were used to linearize DNA for separation by electrophoresis and Southern blotting allowed for the identification of specific DNA segments via hybridization probes. In, Berg utilized restriction enzymes to create the first recombinant DNA molecule and first recombinant DNA plasmid. In, Cohen and Boyer created the first recombinant DNA organism by inserting recombinant DNA plasmids into *E. coli*, now known as bacterial transformation, and paved the way for molecular cloning. The development of DNA sequencing techniques in the late, first by Maxam and Gilbert, and then by Frederick Sanger, was pivotal to molecular genetic research and enabled scientists to begin conducting genetic screens to relate genotypic sequences to phenotypes. Polymerase chain reaction (PCR) using polymerase, invented by Mullis, enabled scientists to create millions of copies of a specific DNA sequence that could be used for transformation or manipulated using agarose gel

separation. A decade later, the first whole genome was sequenced (*Hhemophilic*), followed by the eventual sequencing of the human genome via the Human Genome Project in 2001. The culmination of all of those discoveries was a new field called genomics that links the molecular structure of a gene to the protein or RNA encoded by that segment of DNA and the functional expression of that protein within an organism. Today, through the application of molecular genetic techniques, genomics is being studied in many model organisms and data is being collected in computer databases like NCBI and Ensemble. The computer analysis and comparison of genes within and between different species is called bioinformatics, and links genetic mutations on an evolutionary scale.

Reverse genetics is the term for molecular genetics techniques used to determine the phenotype resulting from an intentional mutation in a gene of interest. The phenotype is used to deduce the function of the un-mutated version of the gene. Mutations may be random or intentional changes to the gene of interest. Mutations may be a mis-sense mutation caused by nucleotide substitution, a nucleotide addition or deletion to induce a frameshift mutation, or a complete addition/deletion of a gene or gene segment. The deletion of a particular gene creates a gene knockout where the gene is not expressed and a loss of function results (e.g. knockout mice). Mis-sense mutations may cause total loss of function or result in partial loss of function, known as a knockdown. Knockdown may also be achieved by RNA interference (RNAi). Alternatively, genes may be substituted into an organism's genome (also known as a transgene) to create a gene knock-in and result in a gain of function by the host. Although these techniques have some inherent bias regarding the decision to link a phenotype to a particular function, it is much faster in terms of production than forward genetics because the gene of interest is already known.

Currently, clinical practice has a fairly wide range of laboratory diagnostic methods that allow not only to diagnose diseases, monitor therapy, but also to monitor treatment. Until recently, laboratory diagnostic methods used in clinical practice had one common drawback - they did not take into account the patient's predisposition to various diseases according to genetic factors. The issues of a patient's predisposition to various diseases are at the heart of a new direction of medicine - personalized medicine, which can be defined as a strategy, prevention and treatment of diseases based on the results of molecular genetic studies.

Thanks to scientific research, it became known that genetic polymorphisms play an important role in the development of various diseases - genome changes occurring in the human population in at least 2 variants (alleles) with a frequency of at least 1%. The most common type of genetic polymorphism is single nucleotide substitutions (SNPs), which are genetically unique for each person. Some polymorphic variants of genes ("predisposition genes") under certain unfavorable conditions can contribute to the development of multifactorial diseases. Unfavorable allelic variants of these genes can cause such frequent diseases as atherosclerosis, coronary heart disease (CHD), osteoporosis, diabetes mellitus, bronchial asthma, tumors, etc. Combinations of allelic variants of various genes that provide a normal metabolic process or are involved in the development of a specific pathology are called "gene networks". The elucidation of the components of the gene network of each multifactorial disease, the development on this basis of a

set of preventive measures for a particular patient form the basis of predictive (predictive) medicine.

Currently, molecular diagnostic technologies are being developed, improved and introduced into clinical practice. Thus, clinical laboratory diagnostics already has a wide range of methods based on the detection and diagnosis of nucleic acid analysis methods - polymerase chain reaction (PCR), genotyping, biochips, sequencing, etc.

PCR is one of the few laboratory diagnostic methods currently used in clinical practice, characterized by the highest specificity and sensitivity in detecting diseases such as bacterial vaginosis, trichomonas's, syphilis, viral hepatitis, HIV infection, tuberculosis, etc.

The PCR method is especially effective in detecting hard-to-cultivate and uncultivated viruses and bacteria, which are often encountered in latent and chronic infections. It should be noted that the diagnostic capabilities of PCR, unlike bacteriological and biological diagnostic methods, are not limited by the ability of microorganisms and viruses to grow on artificial media or in cell culture.

The main advantage of PCR over bacteriological and biological diagnostic methods is the ability to identify, determine the properties and work with a wide variety of microorganisms that cannot be propagated in the laboratory for one reason or another.

Research by the method of polymerase chain reaction can be carried out on the basis of both separately built laboratories and already existing ones. In the latter case, it is necessary to have independent zones that correspond to the PCR stages:

Acceptance and registration of material.

Parsing, sorting and initial processing.

Isolation of DNA or RNA.

Creating reagents and performing PCR.

Trapping of reaction products.

The first two departments of the molecular laboratory can be combined into one, and when using real-time PCR (real-time PCR), there is no need for an electrophoresis zone.

The above are the basic zones, without which the laboratory of molecular diagnostics will not be able to work. For the comfort of the staff and increase its efficiency, it is recommended to add a rest room, a locker room, a kitchen, a toilet, an archive, a utility room - all together or something of the above, at the choice of the customer.

Naturally, there must be electricity, heating, uninterrupted water supply and sewerage. When planning rooms, it is necessary to ensure a continuous flow of material transport and at the same time exclude air exchange between rooms.

Equipment of the molecular diagnostics laboratory

Here, the equipment of a typical PCR laboratory is considered, starting from the zones described in the previous section.

CONCLUSION

Acceptance, registration, sorting and primary processing of incoming material. This area is usually located in a separate boxed room, which allows the incoming material to be properly processed and to perform its initial processing. In addition to laboratory tables/desks and chairs, a centrifuge

is installed to allow sample components to be separated and precipitated properly. If necessary, the centrifuge can be equipped with a cooling or heating option.

Isolation of DNA and RNA. A separate room is allocated for this area in the molecular laboratory, although it can be combined with other types of research, except for genetic engineering. In the latter case, a special box of bacterial air environment for PCR is installed, in which, in no case, other work is allowed!

If the molecular lab is small, the DNA/RNA extraction area can be combined with PCR, again with separate boxes.

As a rule, when extracting DNA / RNA, the following are used:

✔ Centrifuges - separate and sediment samples.

✔ Aspirators - Removal of residual volumes of buffer solution or alcohol.

✔ Bactericidal recirculates - ultraviolet cleaning and disinfection of the surrounding air.

✔ Distillers - continuous production of the required volumes of distilled water.

✔ Thermostats - finding the samples under study in a stable temperature regime.

✔ Nano photometers - determination of the quantitative and qualitative composition of the studied nucleic acids.

✔ Laboratory refrigerators and freezers - short-term or long-term storage of samples and PCR solutions.

Laboratory test tube racks, variable volume dispensers, and test tubes may also be required.

Conducting PCR. To perform a polymerase chain reaction, a molecular laboratory requires the following equipment:

✔ Sterile box - guarantees maximum cleanliness of the environment and protection of samples from contamination.

✔ Thermal cycler - perform PCR.

✔ Minicentrifuge/multivortex - mix and separate samples.

✔ Bactericidal recirculator - disinfection of the ambient air with ultraviolet light.

✔ Refrigerator and freezer - can be combined in one device.

Test tubes, racks and dispensers are also in demand in this area.

Definition of results. To capture PCR products, it is necessary to equip a separate room in the molecular diagnostics laboratory - it is better if it is equipped with a sterile box.

You may also need:

✔ Electrophoresis device - separation of products in agarose gel.

✔ Documentation system - dark box, digital camera, trans illuminator, PC/laptop and printer.

✔ Precision balance - preparation of agar for electrophoresis.

✔ Microwave oven - also needed to prepare agarose gel.

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