Recombinant DNA Technology

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The development of molecular genetic research techniques has provided researchers with the possibility to unify DNA molecules from different sources, i.e. from different organisms, in vitro. DNA molecules formed through unification of molecules from different sources are called recombinant DNA. It is a technology which includes processes in which DNA molecules, which do not occur on their own in nature and which are obtained from mostly different biological species, are cut and the obtained different DNA parts are pasted by genetic engineering techniques. The term "Recombinant DNA", however, is used for the new DNA molecule resulting as a product of this process and is abbreviated as rDNA.

Restriction endonuclease analyses, DNA sequence and genetic analysis and manipulations such as directed mutations can be implemented on recombinant DNA. All the techniques used for these processes are called recombinant DNA technology. This technique is also known as genetic engineering.

The processes made in this field can be summarized as the production of genes by taking them from any kind of organism (cloning) and the use of the produced genes for basic or applied researches. Nowadays, this technology has started to be widely used in areas such as basic sciences, medicine, industry, livestock farming, agriculture, and environmental engineering.

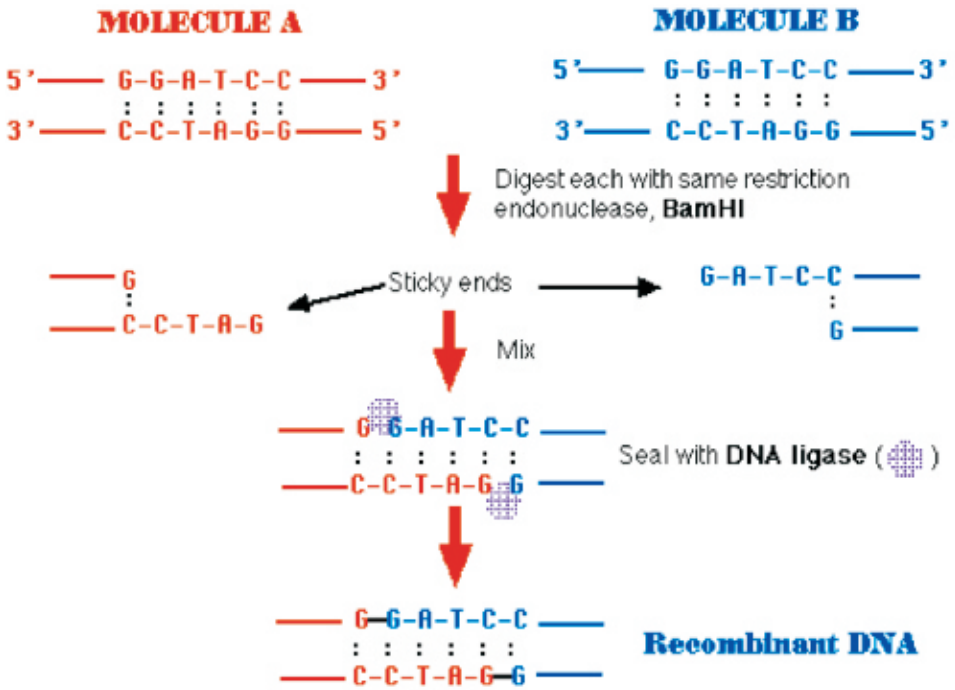
Diversification (recombination), which is the scientific basis of this technology, is a genetic phenomenon and is one of the main reasons for the variation between creatures in nature. Recombination is a series of events in which individuals of different genotypes make reproduction, and the features of the parental generation are newly combined in the second generation. This happens on a molecular level. The homologue regions of the DNA molecules, which differ in their sequence of nucleotides, exchange parts. Thus, there is a breakup between DNA molecules and in these sections, a part exchange takes place between the DNA molecules. As a result, *recombinant* DNA are produced, which do not totally conform to the original DNA molecules and which partly contain their nucleotide sequence.

Homologue variation (Recombination) is a result of reproduction and usually results from chromosomal part exchange during the meiosis. The variation among bacteria can be seen through different processes such as transformation, conjugation and transduction.

The basis of all these processes is the homology between DNA molecules. Because of this, the variation between individuals of the same species or between very similar species is limited. Reproduction obstacles at various levels existing between different species prevent the transfer of genetic information between individuals of different species, and consequently, prevent recombination.

The definition and scope of recombinant DNA varies according to different communities and scientists. Together with this, a broad definition suitable for the modern criteria of our time and unifying the common features of these different definitions can be made as following:

Recombinant DNA Technology is the unit of techniques used for studies in which the gene of a living is put into a suitable host cell where it is aimed to reproduce and sometimes to be defined.



With this technology, which is applied directly on genetic material for a definite aim, genetic material can be modified under in vitro conditions and by putting chosen genes into chosen livings, those can reproduce in this unnatural situation and this technology can define which gene is necessary to obtain the product wished. The species of procaryotic and eukaryotic groups make gene transfer only with species of their own group. With this technology, it is possible to make gene transfer between the species of the two different groups and to produce variation. Transgenic animals are known as animals whose genomes contain a recombinant gene of a different organism.

The rapid development of recombinant DNA technology offers no opportunities to study gene activities. The importance of gene transfer technology is increasing every day through studies made in the field of molecular biology and genetic engineering. Gene transfer on farming animals has the aim to increase the number of offspring, the amount, artificial amount and quality of milk, beneficial use of food and immunity against illnesses. Another important aim is to enable transgenic animals to become organ donor.

When recombinant DNA techniques were more and more developed, it was only used for studies based on academic research, such as on gene organization, sequence and mutations.

Scientists profiting from this technology have also found its industrial application and today under the title of biotechnology an important industry is born which contributes billions of Liras to the economy in different fields such as medicine, cosmetics and agriculture.

By using recombinant DNA technology, the cloning of a gene is carried out in four stages. Gene cloning can be defined as follows: It is the process in which an important product, i.e. the DNA region aimed, is unified with a vector and its copies are obtained in special host cells in high numbers.

1) Gene isolation

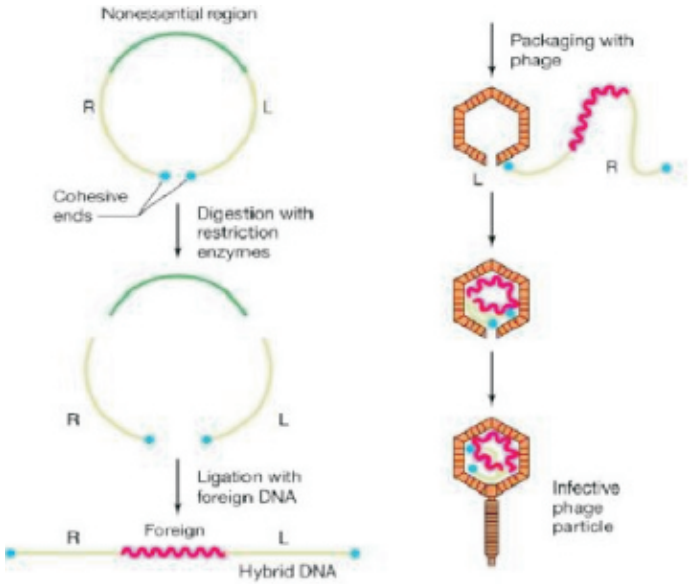
a) the synthesis of complement DNA (cDNA) from its mRNA

b) use of gene libraries: if the labelled copy or labelled mRNA of the searched gene is available, the clone containing the searched gene can be determined among the clones containing the whole genome in parts and can be used in high numbers for gene isolation.

2) a suitable gene carrying tool (Vector)

a) Plasmids: Plasmids are circular molecules which can be found in E. coli host cells separated from small chromosomes (2kb-200kb), the latter of which is available in a high number of copies. The mostly used plasmid pBR322 contains immune areas against ampiciline and tetracycline antibiotics. Having only one cutting place for a lot of restriction enzymes, it can carry foreign DNA parts until 6000bp.

b) Viruses: in the Asimolar conference in 1978, it has been decided to use the least dangerous and most studied virus λ-phage. λ-DNA is a molecule of 49kb height and double roped. On both ends, it has 12 one-roped and nucleotide complement chains. These ends are called "cos" (cohesive). In an infectious cell, the DNA is synthesized one after another in the cos region in form of DNA chains and later packed in phage style.



Cloning DNA in bacteriophage λ

c) cosmids: A cosmid is a hybrid vector containing the usable features of plasmids and phages. It can use DNA parts for cloning until 35,000-45,000bp. It can recognize the "cos" region of enzymes which pack λ phage. The "cos"regions of λphage are cloned e.g. into the tet/r gene pBR322. Amp/r gene stays complete. Foreign DNA is cut into relatively small parts and each of them is combined with plasmids of the "cos" region. Bacteria are replanted. With a little luck, they are chosen according to their resistance to ampicilline and their sensitivity to tetracycline.

d) Expression vectors: they are artificial plasmids consisting of a preferentially promoter region in front of the region where the foreign gene is going to be inserted. Expression vectors are used if it is wished to synthesize the protein which is a product of the gene to be cloned.

3) Insertion of the gene into the cell

a) with plasmids or viruses

b) through chemical methods: DNA is trapped by using Ca_3 $(PO4)_2$ and put into a selected environment (identified with ±tk gene)

c) physical method: DNA injection with glass (or plastic) micro pipettes (ends have to be 0,1-0,5 micron), which have to be let into the cell nucleus. With this method, any DNA part can be directly inserted into any cell.

d) with fusion: By using liposomes or erythrocyte apparitions (erythrocyte membranes whose hemoglobin and other proteins have been emptied out and whose completeness is saved), their DNA is combined with another cell through the cell melting method. Istanbul Medical Faculty, Department of Biophysics

4) Selection of the cell which contains the gene:

a) resistance against antibiotics

B)tk-cells cannot reproduce in selective environment (e.g. HAT: hypotaxine aminopterine, thymine) because aminopterine blocks the way between dCTP and dTDP. After choosing the cell containing the foreign gene, it can be kept under suitable conditions or if it is wished, the gene mentioned can be used like a producing factory. Nowadays, the genes of valuable proteins (insulin, growth hormone, etc.) are available as cloned from expression vectors. They can even be provided from different companies by being sold.

Recombinant DNA technology applications are especially used in genetic engineering and biotechnology. 3-5% of the world population is affected by hereditary diseases which are generally irremediable. The biggest hope and expectation in this area is to repair genetic disorders through gene transfer methods or to develop efficient treatment modalities. The real value of this method, which has found a very wide range of application in a short time, will be better understood within the next years.

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