Rdna Technology Services Section Front Page

Biotechnology is the application of biological organisms, systems, or processes by various industries to learning about the science of life and the improvement of the value of materials and organisms such as pharmaceuticals, crops, and livestock. It is a relatively new and fast-developing field that integrates knowledge from several traditional sciences: biochemistry, chemistry, microbiology, and chemical engineering. The term itself is largely believed to have been coined in 1919 by Hungarian engineer Károly Ereky. In the late 20th and early 21st century, biotechnology has expanded to include new and diverse sciences such as genomics, recombinant gene technologies, applied immunology, and development of pharmaceutical therapies and diagnostic tests. Recombinant DNA molecules are sometimes called chimeric DNA, because they are usually made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms. Proteins that result from the expression of recombinant DNA within living cells are termed recombinant proteins. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced. Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring of the foreign coding sequence. Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

Recombinant DNA

The idea of recombinant DNA was first proposed by Peter Lobban, a graduate student of Prof. Dale Kaiser in the Biochemistry Department at Stanford University Medical School (Lear, 1978) The first publications describing the successful production and intracellular replication of recombinant DNA appeared in 1972 and 1973(Jackson et al., 1973; Lobban and Kaiser, 1973). Stanford University applied for a US patent on recombinant DNA in 1974, listing the inventors as Stanley N. Cohen and Herbert W. Boyer; this patent was awarded in 1980. The first licensed drug generated using recombinant DNA technology was human insulin, developed by Genentech and Licensed by Eli Lilly and CompanyRecombinant DNA (rDNA) molecules are DNA molecules

formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure. They differ only in the nucleotides sequence within that identical overall structure.

Application of Recombinant DNA

Vectors in gene therapy

A recombinant virus is a virus produced by recombining pieces of DNA using recombinant DNA technology. This may be used to produce viral vaccines or gene therapy vectors.

Viruses

All viruses bind to their hosts and introduce their genetic material into the host cell as part of their replication cycle. Therefore this has been recognized as a plausible strategy for gene therapy, by removing the viral DNA and using the virus as a vehicle to deliver the therapeutic DNA. A number of viruses have been used for human gene therapy, including retrovirus, adenovirus, lentivirus, herpes simplex virus, vaccinia, pox virus, and adeno-associated virus.

Recombinant Antibodies

Developments in the fields of bacterial expression of functional antibodies and methods to select genes from a library by using the phenotype of the encoded polypeptide have been a breakthrough in antibody technology. Today, phage display in combination with antibody gene libraries is widely used to select E. coli host cells that express desired antibody fragments. Such gene libraries are typically produced either from natural sources (e.g., from the spleen of an immunized animal or from plasma cells of human donors) or generated by genetic engineering. The latter has been used to create naïve libraries based on one or more antibody VH and VL gene segments that are diversified by cassette mutagenesis or similar approaches.

Such libraries are typically unbiased and can be used for any given antigen (Knappik et al., 2000; Soderlind et al., 2000; Hoet et al., 2005). Modern naïve libraries are generally large (more than 1010 members), contain only few non-functional members, yield antibodies that are well expressed in E. coli (more than 1 mg of purified material per liter of culture) and are designed to allow further affinity maturation, if needed. Phage display is then most often used to select desired antibodies from such libraries.

Recombinant monoclonal antibodies

The production of recombinant monoclonal antibodies involves technologies, referred to as repertoire cloning or phage display/yeast display. Recombinant antibody engineering involves the use of viruses or yeast to create antibodies, rather than mice. These techniques rely on rapid cloning of immunoglobulin gene segments to create libraries of antibodies with slightly different amino acid sequences from which antibodies with desired specificities can be selected. The phage antibody libraries are a variant of the phage antigen libraries first invented by George Pieczenik. These techniques can be used to enhance the specificity with which antibodies recognize antigens, their stability in various environmental conditions, their therapeutic efficacy, and their detectability in diagnostic applications. Fermentation chambers have been used to produce these antibodies on a large scale.

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