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PATENTS AND LITERATURE

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The objective of this section is to keep readers aware of significant inventions and trends in industrial research as well as to highlight those areas of research that may lead to new biotechnological opportunities. In addition to DNA probes for clinical applications covered in the last issue, three other subject areas are being surveyed in 1986: protein engineering, mammalian cell culture, and microbial transformations. The subject of this, the fourth Patents and Literature Section of 1986 is protein engineering and site-directed mutagenesis.

Protein Engineering and Site-Directed Mutagenesis

Patents

This section identifies and gives a brief description of patents from the US patent literature from January 1975 through February 1986. The search headings were protein engineering, site-directed mutagenesis, and nucleotide modification or alteration. Both patent titles and abstracts were searched. Patent assignees were also searched for several of the major biotechnology companies. Copies of the US patents can be obtained for $1.50 each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

Bahl, C. P.
METHOD FOR SINGLE NUCLEOTIDE ALTERATION
US 4,351,901, Sep. 28, 1982
Assignee: Cetus Corp.

A method is described for altering a single nucleotide at a predetermined position in a gene involving the isolation of a single strand gene fragment extending up to the position before the nucleotide to be altered. A ribonucleotide or a protected deoxyribonucleotide corresponding to the desired altered nucleotide is attached at the end of this fragment. The fragment is then annealed to a complementary template that extends beyond the end of the fragment. The fragment is then extended complementary to the remainder of the template. The resulting partially mismatched double-stranded DNA is used to produce a pure DNA gene containing an altered deoxyribonucleotide at the single desired position.

Bender, R. and Duck, P. D.
CHEMICAL SYNTHESIS APPARATUS FOR PREPARATION OF POLYNUCLEOTIDES
US 4,353,989, Oct. 12, 1982
Assignee: ens Bio Logicals Inc.

An apparatus is described for the stepwise synthesis of polynucleotides in which the polynucleotide chains are extended in stepwise fashion from a modified form of polymer support to which the first unit is linked. This apparatus includes a reaction column containing the polymer supported product, which acts as the reaction vessel, and a series of reaction bottles all connected by two-way valves arranged in series to the column by means of a fluid-flow conduit. The farthest upstream vessel of the series contains reaction solvent, used for washing purposes. The most downstream of the reaction vessels contain nucleotide reagents. Each of the values has two separate and discreet fluid-flow passageways, the first used exclusively for flow of reagent from the vessel and the second for flow of solvent or reagents from an upstream vessel. This eliminates the possibility of cross-contamination as a result of residual reagent left in the dead space of the valve. The valves are biased toward their solvent flow condition. Materials are drawn through the fluid flow conduit and values by suction.

Itakura, K.
RECOMBINANT DNA CLONING VEHICLE
US 4,356,270, Oct. 26, 1982

and

MAMMALIAN GENE FOR MICROBIAL EXPRESSION
US 4,571,421, Feb. 18, 1986
Assignee: Genetech, Inc.

Recombinant microbial cloning vehicles comprising heterologous DNA coding for the expression of mammalian hormones and other polypeptides, including plasmids suited for the transformation of bacterial hosts, are described. The latter incorporate a regulon homologous to the host in its untransformed state, in reading phase with the structural gene for the heterologous DNA. Cloning vehicles are described that code for the microbial expression of various proteins, including (a) a polypeptide hapten and additional protein sufficient in size to confer immunogenicity on the product of expression, which may find use in raising antibodies to the hapten for assay use or in the manufacture of vaccines; and (b) a desired polypeptide product and additional protein from which the desired product may be cleaved. Methods of preparing synthetic structural genes coding for the expression of mammalian polypeptides in microbial cloning systems are also described.

Mark, D. F., Lin, L. S., and Lu, S. D. Y.
HUMAN RECOMBINANT INTERLEUKIN-2 MUTEINS
US 4,518,584, May 21, 1985
Assignee: Cetus Corp.

Muteins of biologically active proteins, such as IFN-Beta and IL-2, in which cysteine residues that are not essential to biological activity have been deleted or replaced with other amino acids to eliminate sites for intermolecular crosslinking or incorrect intramolecular disulfide bridge formation. These muteins are made by bacterial expression of mutant genes that encode the muteins that have been synthesized from the genes for the parent proteins by oligonucleotide-directed mutagenesis.

Paau, A., Platt, S. G., and Sequeira, L.
ASSAY METHOD AND PROBE FOR POLYNUCLEOTIDE SEQUENCES
US 4,556,643, Dec. 3, 1985
Assignee: Agracetus

An assay method for the detection of a specific nucleotide target sequence in a polynucleotide test extract is disclosed that utilizes a polynucleotide-modified probe, including both a cDNA sequence substantially complementary to the specific target sequence and a protein binding sequence. The assay is conducted by exposing the modified probe to the polynucleotide test extract for hybridization and then exposing the complex to the protein that binds to the protein-binding sequence. An assay, such as an immunoassay, is then conducted on the test sample to indicate the presence of the specific target sequence by detecting the presence of the binding protein.

Paddock, G. V.
RECOMBINANT cDNA CONSTRUCTION METHOD AND HYBRID NUCLEOTIDES USEFUL IN CLONING
US 4,362,867, Dec. 7, 1982
US 4,503,151, Mar. 5, 1985
Assignee: Research Corp.

Compounds useful as complementary DNA (cDNA), including deoxyribonucleotides and at least one ribonucleotide, are described. They may be depicted by the general formula: 3′(rN) (*b*) (dN) (*a*) 5′5′ (dN) (*c*) 3′, where (dN) (*a*) and (dN) (*c*) represent series of deoxyribonucleotides and (rN) (*b*) represents a series of ribonucleotides; *a*, *b*, and *c* are the number of nucleotides in the series, and *b* is ≥ 1, *a* is ≥ 35, and *c* is ≥ 10; the series of deoxyribonucleotides (dN) (*a*) includes a series of deoxyribonucleotides that are substantially complementary to the series of deoxyribonucleotides (dN) (*c*). These compounds may be prepared from messenger RNA (mRNA) containing the genetic information necessary for cellular production of desired products, such as polypeptides. After appropriate modification, they may be combined with DNA from a suitable cloning vehicle, such as a plasmid, and the resulting combined DNA used to transform bacterial cells. The transformed bacterial cells may then be grown and harvested and the desired product or products recovered.

Riggs, A. D.
METHOD FOR MICROBIAL POLYPEPTIDE EXPRESSION
US 4,366,246, Dec. 28, 1982

and

MICROBIAL POLYPEPTIDE EXPRESSION VEHICLE
US 4,425,437, Jan. 10, 1984

and

TRANSFORMANT BACTERIAL CULTURE CAPABLE OF EXPRESSING HETEROLOGOUS PROTEIN
US 4,431,739, Feb. 14, 1984

and

METHOD AND MEANS FOR SOMATOSTATIN PROTEIN CONJUGATE EXPRESSION
US 4,563,424, Jan. 7, 1986
Assignee: Genetech, Inc.

Recombinant microbial-cloning vehicles comprising heterologous DNA coding for the expression of mammalian hormones and other polypeptides, including plasmids suited for the transformation of bacterial hosts are described. The latter incorporate a regulon homologous to the

host in its untransformed state, in reading phase with the structural gene for the heterologous DNA. Cloning vehicles are described that code for the microbial expression of various proteins, including (a) a polypeptide hapten and additional protein sufficient in size to confer immunogenicity on the product of expression, which may find use in raising antibodies to the hapten for assay use or in the manufacture of vaccines; and (b) a desired polypeptide product and additional protein from which the desired product may be cleaved. Methods of preparing synthetic structural genes coding for the expression of mammalian polypeptides in microbial cloning systems are also described.

Rubin, H.
METHOD OF INSERTING AMINO ACID ANALOGS INTO PROTEINS
US 4,568,640, Feb. 04, 1986

A method is described for substituting one amino acid for another in a protein chain to improve selected properties of the protein. Initially, an mRNA molecule capable of producing an unmodified protein of the type it is desired to modify is obtained. Next, the codon along the mRNA chain that ordinarily accepts the anticodon corresponding to a first amino acid that is to be replaced with a selected second amino acid is determined. The tRNA that ordinarily brings the first amino acid to that site is modified to carry the second amino acid instead. The modified protein is then formed by translation techniques in the presence of the modified tRNA, whereby the second amino acid is incorporated in the protein chain in place of the first amino acid. Several methods of accomplishing the modification of the selected tRNA are also described.

Weissman, S. M., Pereira, D., and Sood, A.
METHOD FOR CLONING GENES
US 4,394,443, Jul. 19, 1983
Assignee: Yale University

A method is provided for isolating and identifying a recombinant clone having a DNA segment coding for at least one desired heterologous polypeptide, at least a short amino acid sequence of which is known. cDNA synthesis is performed on a mixture of mRNAs containing the mRNA coding for the desired polypeptide, the resultant cDNA mixture is isolated, inserted into recombinant cloning vehicles, the hosts are transformed with the vehicles, the transformants are separated, and a recombinant clone is isolated and identified that contains a DNA segment that is at least partially homologous to one oligonucleotide probe specific for the DNA segment. The probe is an extension of the nucleotide sequence of an oligonucleotide primer having a nucleotide sequence complementary to a region of the target mRNA coding for a portion of the known amino acid sequence and is complementary to a

longer region of the target mRNA coding for a longer portion of the known amino acid sequence. Recombinant clones coding for human histocompatibility antigens are described.

Literature

This section surveys literature in the area of protein engineering and site-directed mutagenesis from January 1984 to April 1986. This section includes only selected articles that appeared during this time period.

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