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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 applied immunology covered in the last issue, four other subject areas are being surveyed in 1985: immobilized biocatalysts, nucleic acid technology, affinity separation, and bioassays. The subject of this, the third, Patent and Literature Section of 1985 is Nucleic Acid Technology.

Nucleic Acid Technology

Patents

This section identifies and gives a brief description of patents from the US patent literature from January 1984 through April 1985. The search headings were Recombin . . . and Genetic Vectors. Both patent titles and abstracts were searched. Patent assigness were also searched for several of the major biotechnology companies. Copies of the US patents can be obtained for $1.00 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 Corporation

A method 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 to 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.

Bell, G., Pictet, R., Goodman. H. M., and Rutter, W. J.
TRANSFER VECTOR AND TRANSFORMED MICROORGANISM CONTAINING HUMAN PROINSULIN AND PRE-PROINSULIN GENES
US 4,431,740, Feb. 14, 1984
Assignee: The Regents of the University of California

A DNA having a base of sequence coding for human proinsulin and a DNA having a base sequence coding for human pre-proinsulin have been cloned, and novel recombinant DNA transfer vectors containing cloned DNAs have been constructed. Novel microorganisms transformed by recombinant transfer vectors have been obtained. Certain of these transformed microorganisms have demonstrated capability to express the cloned DNAs, synthesizing human proinsulin and human pre-proinsulin.

Chang, S., and Meade, J. H.
CHIMERIC PLASMIDS THAT REPLICATE IN BACTERIA AND YEAST AND MICROORGANISMS TRANSFORMED THEREWITH
US 4,477,571, Oct. 16, 1984
Assignee: Cetus Corporation

Chimeric plasmids capable of transforming bacteria and yeast that carry the Cm (chloramphenicol resistance) gene and the Tc (tetracycline resistance) gene as selectable markers are described. The Cm gene allows the plasmids to be selected for in wide-type strains of the yeast *Saccharomyces cerevisiae*. The Tc gene allows heterologous genes cloned into the plasmids to be selected for in *Escherichia coli* bacteria.

Cohen, S. N.
METHOD FOR SYNTHESIZING DNA SEQUENTIALLY
US 4,293,652, Oct. 6, 1981
Assignee: Cetus Corporation

A method and a DNA linker are described for synthesizing relatively long double-stranded deoxyribonucleic acid sequences of defined composition. Short complementary single strand segments of

oligonucleotides comprising part of the full sequence desired are synthesized using known procedures. Overlapping single-strand segments are annealed forming double-stranded fragments that are inserted in cloning vectors and cloned in an appropriate host, both purifying the DNA fragments and amplifying the amount. An adjacent fragment is then similarly synthesized in quantity and inserted adjacent to the first synthetic introduced fragments in the cloning vectors. This is followed by cloning in an appropriate host. The procedure continues until the entire desired sequence has been formed, at which time it may be excised or cloned directly in the vectors upon which it was made. The described DNA linker contains a restriction enzyme recognition sequence for a restriction enzyme that cuts offset from the recognition sequence and exactly at the end of the linker, enabling synthesized DNA to be inserted at the end of the linker without disturbing the recognition sequence.

Colby, C. Jr., and Denney, D. W.
INTERFERON PRODUCTION
US 4,262,090, Apr. 14, 1981
Assignee: Cetus Corporation

A method is described for preparing interferon, mRNA for interferon, and competent recombinant DNA containing dsDNA and cDNA from mRNA coding for mammalian interferon. The method employs crossing a mutant mammalian cell that is semiconstitutive for inferon with a cell derived from the same or different mammal having wild-type gene(s) for interferon and for the regulation of interferon synthesis and desirably having phenotypic properties allowing for selection of the hybrid cells. The desired hybrid clones are then induced to produce IF mRNA. The amounts of mRNA for interferon are greatly enhanced over the amounts normally obtained from wild-type cell strains. The mRNA is employed to produce cDNA that codes for the mammalian interferon. The single-stranded cDNA is employed with a replicon recognized by a microorganism host to provide a recombinant DNA. The microorganism host is transformed with the recombinant DNA, so as to provide a source for the interferon gene, as well as interferon.

Colson C. A., Cornelis, P. E., Digneffe, C. S., and Walon, C.
GENETICALLY ENGINEERED MICROORGANISMS FOR MASSIVE PRODUCTION OF AMYLOLYTIC ENZYMES AND PROCESS FOR PREPARING SAME
US 4,469,791, Sep. 4, 1984
Assignee: CPC International Inc.

Genetically engineered microorganisms containing recombinant DNA with an amylase coding gene afforded improved yields of amylase enzymes.

Crea, R.
NUCLEOSIDIC PHOSPHORYLATING AGENT AND METHODS
US 4,393,010, Jul. 12, 1983
Assignee: Genentech, Inc.

Compounds containing a base-labile substituted-phenyloxy protecting group, are useful as phosphorylating reagents in the synthesis of oligonucleotides by a phosphotriester method.

Crea, R.
NUCLEOSIDIC PHOSPHORYLATING AGENT AND METHODS
US 4,310,662, Jan. 12, 1982
Assignee: Genentech, Inc.

Compounds containing a base-labile substituted-phenyloxy protecting group, are useful as phosphorylating reagents in the synthesis of oligonucleotides by a phosphotriester method. Such compounds are made by the reaction of B-cyanoethanol and a corresponding R-phosphorodichloridate. A method of phosphorylating involves reacting such compounds, in the presence of base, with a nucleoside or a polynucleotide having a free 3′-hydroxyl group, having the 5′-O position protected by an organic group labile in acidic medium and having any phosphotriester linking groups protected by an organic group labile in strong basic medium.

Curtiss, R. III
MODIFIED MICROORGANISMS AND METHODS OF PREPARING AND USING SAME
US 4,190,495, Feb. 26, 1980
Assignee: Research Corporation

Microorganisms have been developed that may be characterized as possessing substantially all of the following qualities or capabilities: (a) capable of accepting foreign genetic information and with its expression permitting the production and recovery of useful gene products; (b) the microorganism being dependent for growth and survival upon defined conditions; (c) the microorganism being incapable of establishment of growth or colonization and/or survival under conditions or in ecological niches that are considered to be natural and/or undesirable for it; (d) the microorganism being capable of causing genetic information incorporated within it to undergo degradation under conditions or in ecological niches that are considered to be natural and/or undesirable for it; (e) the microorganism being capable of permitting cloning vectors incorporated within it to be dependent for their replication, maintenance, and/or function of the microorganism; (f) the microorganism being substantially imcapable of transmitting cloning vectors of recombinant DNA molecules incorporated within it to other organisms under conditions or ecological niches that are considered to be natural and/or undesirable for it;

(g) the microorganism being capable of being monitored by suitable means and/or techniques without substantial alteration of the microorganism; and (h) the microorganism being susceptible of substantially minimal contamination with other organisms when recombinant DNA molecules are incororated within it and being incapable of contaminating the other organisms. Examples of such microorganisms include various strains of *Escherichia coli.* Additionally, techniques have been developed and employed for imparting special properties, e.g., genetic properties, to microorganisms that render the resulting microorganisms unique. Also techniques have been developed for the handling of plasmid and/or bacteriophage cloning DNA vectors for eventual insertion into microorganisms for testing and techniques have been developed for the transformation of microorganisms, for the introduction of recombinant DNA molecules into these microorganisms. Also, techniques have been developed in connection with the development or production of these microorganisms that impart special genetically linked properties to them.

Dean, D. H., and Ellis, D. M.
AMYLASE-NEGATIVE, ASPOROGENOUS MUTANT OF *Bacillus subtilis* USEFUL AS A HOST IN A HOST–VECTOR SYSTEM.
US 4,465,773, Aug. 4, 1984
Assignee: CPC International Inc.

A biologically pure amylase-negative, asporogenous mutant *B. subtilis* (ATCC 39,096) is provided. This host, which contains no amylase-coding gene, is particularly useful as a host in a host-vector system for recombinant DNA work directed toward the production of improved strains of amylase-producing microorganisms.

Dean, D. H., and Ellis, D. M.
ASPOROGENIC MUTANT OF *Bacillus subtilis* USEFUL AS A HOST IN A HOST–VECTOR SYSTEM
US 4,450,236, May 22, 1984
Assignee: CPC International Inc.

A biologically pure strain of asporogenous *B. subtilis* (ATCC 39,095) is provided. This strain, which shows a low frequency of reversion to spore formers, is suitable for a host providing a high level of biological containment in a host–vector system for use in recombinant DNA methology.

Dean, D. H., and Ellis, D. M.
ASPOROGENIC MUTANT OF *Bacillus subtilis* USEFUL AS A HOST IN A HOST–VECTOR SYSTEM
US 4,450,235, May 22, 1984
Assignee: CPC International Inc.

A biologically pure strain of asporogenous *B. subtilis* (ATCC 39,094) is provided. This strain, which shows a low frequency of reversion to spore

formers, is suitable for a host providing a moderate to high level of biological containment in a host-vector system for use in recombinant DNA methodology.

Fayerman, J. T., and Richardson, M. A.
CLONING VECTORS FOR USE IN STREPTOMYCES AND RELATED ORGANISMS
US 4,513,086, Apr. 23, 1985
Assignee: Eli Lilly and Company

A selectable recombinant DNA cloning vector for use in *Streptomyces* and related organisms is disclosed.

Foor, F., and Morin, N. R.
DNA CLONING VECTOR TG1, DERIVATIVES, AND PROCESSES OF MAKING
US 4,460,689, Jul. 17, 1984

AND

Foor, F., and Roberts G. P.
BACTERIOPHAGE DNA CLONING VECTOR TG1 AND MICROORGANISMS CONTAINING TG1
US 4,508,826, Apr. 02, 1985
Assignee: Merck & Co Inc.

A novel bacteriophage (TG1, TG1 derivatives) and the corresponding genome or nucleic acid components are disclosed. These are useful as DNA cloning vectors into organisms such as the bacterium *Streptomyces catlleya;* portions of such phage genome are additionally useful as adjuncts in recombinant DNA cloning procedures, for example: (1) to permit the maintenance of cloned DNA in the host, either in an integrated state or as an autonomous element; (2) to serve as promoters of the expression of endogenous or foreign genes where they are ligated to such genes or otherwise service as promoters; and (3) to serve as regulatory elements for achieving control over endogenous and foreign gene expression; as cloning vectors, TG1, its deletion mutants, and other derivatives serve for the amplification and transfer of DNA sequences (genes) coding for useful functions, and of useful DNA sequences for example, as distinct plasmic vectors; such modified cloning vectors (hybrid DNA molecules comprising all or portions of the TH1 genome and foreign DNA sequence) are introduced into the recipient organism by infection, transfection, or transformation; in which the hybrid DNA functions in an integrated mode, in a lytic (vegetative phase) mode, and/or in a plasmid mode. Microorganisms comprising TG1 prophage and deletion and hybrid (chimeric) derivatives; and microorganisms comprising hybrid (chimeric) phage-plasmids and derivatives are also disclosed.

Goeddel, D. V.
HYBRID HUMAN LEUKOCYTE INTERFERONS
US 4,456,748, Jun. 26, 1984
Assignee: Genentech, Inc.

AND

Goeddel, D. V.
HYBRID HUMAN LEUKOCYTE INTERFERONS
US 4,414,150, Nov. 08, 1983
Assignee: Genentech, Inc.

Methods of microbially preparing novel human hybrid leukocyte interferons, useful in the treatment of viral and neoplastic diseases, by DNA recombination of parental interferon genes, taking advantage of common restriction endonuclease cleavage sites therein and in carrier expression plasmids, are disclosed.

Goeddel, D. V., and Heyneker, H. L.
METHOD OF CONSTRUCTING A REPLICABLE CLONING VEHICLE HAVING QUASISYNTHETIC GENES
US 4,342,832, Aug. 03, 1982
Assignee: Genentech, Inc.

Methods for the construction and microbial expression of quasisynthetic genes arising from the combination of organic synthesis and enzymatic reverse transcription from messenger RNA sequences incomplete from the standpoint of the desired protein product. Preferred products of expression lack bioinactivating leader sequences common in eukaryotic expression products, but problematic with regard to microbial cleavage to yield bioactive material. A gene coding for human growth hormone, a preferred embodiment, is constructed and expressed.

Goodman, H. M., Shine, J., and Seeburg, P. H.
MICROORGANISM CONTAINING GENE FOR HUMAN CHORIONIC SOMATOMAMMOTROPIN
US 4,447,538, May 08, 1984
Assignee: Regents of the University of California

A microrganism containing a recombinant DNA transfer vector having the coding sequences for human chorionic somatomammotropin is disclosed.

Hamer, D. H., and Gerin, J.
SIMIAN VIRUS RECOMBINANT THAT DIRECTS THE SYNTHESIS OF HEPATITIS B SURFACE ANTIGEN
US 4,442,205, Apr. 10, 1984

Assignee: The United States of America as represented by the Department of Health and Human Services

A process for producing a recombinant between simian virus 40 (SV40) and hepatitis B virus (HBV) is given. When tissue culture cells are infected with the recombinant, hepatitis B surface antigen is produced. Because a single cloned gene is used, the surface antigen produced is homogeneous and can be produced without conventional dependence on human sera. The antigen is excreted into the culture medium as 22 nm particles with the same physical properties, antigenic composition, and constituent polypeptides as those found in the sera of patients with Type B hepatitis. The antigen is useful for the preparation of vaccines.

Hanisch, W. H., and Fernandes, P. M.
PROCESS FOR THE RECOVERY OF HUMAN BETA-INTERFERON-LIKE POLYPEPTIDES
US 4,462,940, Jul. 31, 1984
Assignee: Cetus Corporation

An improved process for recovering and purifying β-HIFN from transformed bacterial. The bacteria are concentrated, disrupted, and the β-HIFN solubilized into an aqueous medium; the β-HIFN is extracted from the aqueous medium with 2-butanol, 2-methyl-2-butanol, or their mixtures; the β-HIFN is precipitated and isolated from the alcohol phase and purified by chromatography and diafiltering the β-HIFN against distilled water or aqueous solutions of ethanol or glycerol at pH of 12; and therapeutic formulations and compositions of β-HIFN in SDS are reduced to less than 10 ppm.

Hershberger, C. L., Radue, A. K., and Rostek, P. R. Jr.
STABILIZING AND SELECTING RECOMBINANT DNA HOST CELLS
US 4,506,013, Mar. 19, 1985
Assignee: Eli Lilly and Company

A method for stabilizing and selecting host cells containing recombinant DNA that expresses a functional polypeptide and the novel organisms and cloning vectors are described. A simple, convenient, and inexpensive method to lyse host cells for purification of intracellular products is also described.

Hershberger, C. L., and Rosteck, Paul R. Jr.
METHOD FOR STABILIZING AND SELECTING RECOMBINANT DNA-CONTAINING HOST CELLS
US 4,436,815, Mar. 13, 1984
Assignee: Eli Lilly and Company

An improved method for stabilizing and selecting host cells containing recombinant DNA that expresses a functional polypeptide and the novel organisms and cloning vectors are disclosed.

Itakura, K.
RECOMBINANT DNA CLONING VEHICLE
US 4,356,270, Oct. 26, 1982
Assignee: Genetech, Inc.

Disclosed are: 1. 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. The latter incorporate a regulon homologous to the host in its untransformed state, in reading phase with the structural gene for the heterologous DNA; 2. Cloning vehicles coding for the microbial expression of a protein comprising 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 a desired polypeptide product and additional protein from which the desired product may be cleaved; and 3. Methods of preparing synthetic structural genes coding for the expression of mammalian polypeptides in microbial cloning systems.

Kikuchi, M., Hayakawa, T., and Kida, M.
PLASMID AND PRODUCTION THEREOF
US 4,478,937, Oct. 23, 1984

A novel plasmid pATM2 is obtained from a microorganism belonging to the genus *Micromonospora,* which is useful as a cloning vector in recombinant DNA work, is disclosed.

Konrad, M. W., and Lin, L. S.
PROCESS FOR RECOVERING HUMAN β-IFN FROM A TRANSFORMED MICROORGANISM
US 4,450,103, May 22, 1984
Assignee: Cetus Corporation

A process for recovering β-IFN from transformed bacteria by disrupting the cell membranes of the bacteria; solubilizing the β-IFN from the disruptate into an aqueous medium with a solubilizing agent such as sodium dodecyl sulfate; extracting it from the aqueous medium with 2-butanol, 2-methylbutanol, or mixtures under conditions that maintain phase separation between the aqueous medium and the extractant; and isolating the β-IFN from the extractant such as by precipitating the β-IFN from an aqueous buffer mixture of the extractant by lowering the pH.

Konrad, M. W., and Mark, D. F.
BACTERIAL PRODUCTION OF HETEROLOGOUS POLYPEPTIDES UNDER THE CONTROL OF A REPRESSIBLE PROMOTER–OPERATOR
US 4,499,188, Feb. 12, 1985

Assignee: Cetus Corporation

A process for bacterially producing heterologous polypeptides, particularly those such as human β-IFN that inhibit bacterial growth, in which bacteria that have been transformed to express the heterologous polypeptide under the control of a trp promotor–operator are cultivated in a known volume of medium containing an excess of a preferred carbon source and a predetermined amount of tryptophan that corresonds approximately to the amount of tryptophan contained in the bacteria. The expression of the heterologous polypeptide is substantially repressed until the bacteria grow to the predetermined elevated cellular density and is then automatically derepressed to permit expression of the heterologous polypeptide.

Kung, H. S.
METHOD FOR THE EXTRACTION OF IMMUNE INTERFERON
US 4,476,049, Oct. 09, 1984
Assignee: Hoffmann-La Roche Inc.

A method for the extraction of intact recombinant human immune interferon with guanidine-HCl is described that permits its purification to homogenity.

Kurahashi, O., Tsuchida, T., Kawashima, H., Enei, H., and Nakamoris, S.
METHOD FOR PRODUCING L-HISTIDINE BY FERMENTATION
US 4,504,581, Mar. 12. 1985
Assignee: Ajinomoto Company Inc.

L-Histidine producing microorganisms that have been constructed by introducing a recombinant plasmid DNA inserted on a chromosomal DNA fragment into a recipient strain of the genus *Bacillus.* These microorganisms are employed to produce L-Histidine in higher than normal yields. The recombinant plasmid DNA inserted is obtained from a donor mutant strain of *B. subtilis* which is resistant to certain L-histidine antagonists. The resistant plasmid confers the properties of the mutant strain upon the recipient *B. subtilis* to make its high yield of product even higher.

Malin, M. E., Fayerman, J. T., Jones, M. D., Mabe, J. A., and Nakatsukasa, W. M.
VECTORS FOR CLONING IN STREPTOMYCES
US 4,468,462, Aug. 28, 1984
Assignee: Eli Lilly and Company

This invention describes selectable recombinant DNA cloning vectors for use in *Streptomyces.*

Miller, J. R., Fayerman, J. T., Kovacevic, S., and Beerman, N. E.

MULTIFUNCTIONAL CLONING VECTORS FOR USE IN *Streptomyces, Bacillus,* AND *E. coli*
US 4,503,155, May 05, 1985
Assignee: Eli Lilly and Company

This invention describes multifunctional recombinant DNA cloning vectors for use in *Streptomyces, Bacillus,* and *E. coli.* The invention further describes transformants of these vectors.

Nakatsukasa, W. M., and Mabe, J. A.
FUNCTIONALLY INDEPENDENT CLONING VECTORS FOR USE IN STREPTOMYCES
US 4,513,085, Apr. 23, 1985
Assignee: Eli Lilly and Company

This invention describes functionally independent selectable recombinant DNA cloning vectors for use in *Streptomyces.*

Olsen, R. H.
GENE SPLICING METHOD AND PRODUCTS PRODUCED THEREFROM
US 4,508,823, Apr. 02, 1985
Assignee: Microlife Technics, Inc.

An improved method of gene splicing and recombinant plasmid transformation is described. The method includes mechanical fragmenting of chromosomal DNA followed by conventional digestion with a restriction enzyme and gene splicing into a vector to provide recombinant plasmids in a bank of at least about 100 different plasmids. The plasmids in the bank are provided for transformation into a suitable host, particularly a plasmid-free bacterium of the same species from which the chromosomal DNA or the vector is derived. The method provides high transformation frequencies because of the presence of multiple "super coiled" or coiled recombinant plasmids in the bank. The method also allows for the direct selection of many different phenotypic traits in a pool of the transformed hosts. The selected hosts are useful for the production of various gene products.

Olsen, R. H.
MOLECULAR CLONING VECTORS FOR USE IN GRAM-NEGATIVE BACTERIA
US 4,508,827, Apr. 02, 1985
Assignee: Microlife Technics, Inc.

Improved cloning vectors derived from pRO1614 are described. One of these vectors, pRO1727, is suitable for cloning using DNA cleaved with the restriction endonuclease, Pstl, and allows selection for the recovery of recombinant plasmids using tetracycline resistance. The cloning

efficiency observed for pRO1727 is higher than described previously for pRO1614 and the host range of this vector is now restricted to *Pseudomonas* bacteria. Another vector, designated pRO1729, is described and developed from pRO1727 by deletion of a portion of its DNA and incorporation of a segment of DNA that encodes for resistance to the antibiotic, chloramphenicol. The chloramphenicol resistance determinant has a cleavage site for restriction endonuclease EcoRI within its chloramphenicol resistance determinant. DNA cloned into this site results in the loss of chloramphenicol resistance, which loss can be detected subsequent to a cloning experiment. Both pRO1727 and pRO1729 are more useful in *Pseudomonas* for cloning than pRO1614.

Paddock, G. V.
RECOMBINANT cDNA CONSTRUCTION METHOD AND HYBRID NUCLEOTIDES USEFUL IN CLONING
US 4,503,151, Mar. 05, 1985
Assignee: Research Corporation

Compounds useful as complementary DNA (cDNA) include deoxyribonucleotides and at least one ribonucleotide. 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; where a, b, and c are the number of nucleotides in the series; where the series of deoxyribonucleotides (dN)(a) includes a series of deoxyribonucleotides that is 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.
TRANSFORMANT BACTERIAL CULTURE CAPABLE OF EXPRESSING HETEROLOGOUS PROTEIN
US 4,421,739, Feb. 14, 1984

AND

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

AND

Riggs, A. D.

METHOD FOR MICROBIAL POLYPEPTIDE EXPRESSION
US 4,366,246, Dec. 28, 1982
Assignee: Genentech, Inc.

Disclosed are: *1.* 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. The latter incorporate a regulon homologous to the host in its untransformed state, in reading phase with the structural gene for the heterologous DNA; *2,* Cloning vehicles coding for the microbial expression of a protein comprised of a polypeptide hapten and additional protein sufficient in size to confer immunogenicity, which may find use in raising antibodies to the hapten for assay use or in the manufacture of vaccines; and a desired polypeptide produce and additional protein from which the desired product may be cleaved; and 3. Methods of preparing synthetic structural genes coding for the expression of mammalian polypeptides in microbial cloning systems.

Rutter, W. J., Goodman, H. M., Ullrich, A., Shine, J., Chirgwin, J., and Pictet, R.
METHOD FOR PRODUCING RECOMBINANT BACTERIAL PLASMIDS CONTAINING THE CODING SEQUENCES OF HIGHER ORGANISMS
US 4,440,859, Apr. 03, 1984
Assignee: The Regents of the University of California

A microorganism having a gene derived from a higher organism is produced by isolating cells from a higher organism containing messenger RNA, extracting the messenger RNA, synthesizing a double-stranded cDNA using the messenger RNA as a template, inserting the cDNA into a plasmid, and transforming a microorganism with the resultant recombinant plasmid.

Schnepf, H. E., and Whiteley, H. R.
Bacillus thuringiensis CRYSTAL PROTEIN IN *Escherichia coli*
US 4,448,885, May 15, 1984
Assignee: Board of the Regents of the University of Washington

Expression of the crystal protein of *B. thuringiensis* in *E. coli* by use of plasmids containing heterologous DNA coding for the crystal protein is described. Genetically engineered bacterial host strains transformed by the recombinant plasmids express *B. thuringiensis* crystal proteins.

Seeburg, P. H.
METHOD FOR CLONING HUMAN GROWTH HORMONE VARIANT GENES
US 4,446,235, May 01, 1984
Assignee: Genentech, Inc.

A novel non-allelic human growth hormone variant is prepared by a new method of obtaining cDNA from the genomic sequences of a eukaryotic organism.

Shizuya, H.
ADENOSINE PYROPHOSPHATE POLYNUCLEOTIDE COMPOUND
US 4,472,572, Sep. 18, 1984
Assignee: Innovax Laboratories, Ltd.

Composition and methods of production of P(1)-adenosine, P(2)-(3′-nucleotide monophosphate)-5′-pyrophosphate are described. AppNp is useful in the synthesis of a predetermined sequence of RNA using the enzyme T4 RNA ligase. The use of this invention in the field of recombinant DNA will greatly enhance the ability to synthesize genes of known composition.

Uhlin, B. E., Nordstrom, K., and Molin, S.
BACTERIA CARRYING PLASMID HAVING TEMPERATURE-DEPENDENT PLASMID COPY NUMBER
US 4,499,189, Feb. 12, 1985

AND

Uhlin, B. E., Nordstrom, K., Molin, S., and Gustaffson, P.
PLASMID HAVING TEMPERATURE DEPENDENT PLASMID COPY NUMBER
US 4,487,835, Dec. 11, 1984
Assignee: A/S Alfred Benzon

High yields of gene products of plasmid DNA, such as proteins, are prepared by cultivating bacteria carrying a plasmid that shows a controlled constant plasmid copy number at one temperature and a much higher or totally uncontrolled copy number at a different temperature. The plasmid may be prepared by recombinant DNA technique using a cloning vector showing the temperature-dependent plasmid copy number pattern.

Ward, C. B., and Bruner, R. D.
METHOD FOR CLONING FILAMENTOUS MICROORGANISMS
US 3,950,224, Apr. 13, 1976
Assignee: Cetus Corporation

A method is described for cloning filamentous microorganisms where the microorganisms are suspended in a liquid medium and fragmented to produce a substantial increase in the number of viable filaments of less than a predetermined size. Those filaments that are smaller than the predetermined size are then separated from the balance of the suspension of filaments by filtering.

Literature

This section surveys literature in the area of Nucleic Acid Technology published from January 1984 to April 1985. This section includes only selected review articles that appeared during this time period under the search headings: recombination, genetic or DNA insertion elements, and gene conversion or genetic vectors.

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