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WO 00/77181 A3

(54) Title: DNA MANIPULATION METHODS, APPLICATIONS FOR SYNTHETIC ENZYMES AND USE FOR POLYKETIDE PRODUCTION

(57) Abstract: The invention comprises a method of assembling several DNA units in sequence in a DNA construct and all derivatives of this method. In particular the production of synthetic enzymes is contemplated. Each DNA unit is provided with the same restriction enzyme recognition site at its 5' and 3' ends. The restriction recognition site at its 3' end being combined with a recognition site for a DNA modification enzyme. A DNA construct having the same or a compatible accessible restriction site, as provided in the DNA unit, is cleaved at the restriction site by the appropriate restriction enzyme. The desired DNA unit is then inserted into the DNA construct, this ligated product subsequently being brought into contact with a DNA modification enzyme such that the restriction site at the 3' end of the inserted DNA unit is abolished. The ligated product is then cleaved at the remaining unmodified restriction recognition site and a subsequent DNA unit is inserted. This process is repeated introducing each desired DNA unit to give a DNA construct containing all the desired units in sequence.

INTERNATIONAL SEARCH REPORT

Inte. ional Application No
PCT/GB 00/02286

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12N15/66 C12N15/52 C12N15/90 C12P17/06
C12P17/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CAB Data, STRAND, EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 17811 A (CHROMAXOME CORP) 30 April 1998 (1998-04-30) claims 1-26; figure 5E; example 5.5.5. ---	22-26
X	ROWE C J ET AL: "Construction of new vectors for high-level expression in actinomycetes" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 216, no. 1, August 1998 (1998-08), pages 215-223, XP004149299 ISSN: 0378-1119 cited in the application the whole document --- -/--	22-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 February 2001	27.02.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 49315 A (KOSAN BIOSCIENCES INC ;UNIV LELAND STANFORD JUNIOR (US)) 5 November 1998 (1998-11-05) claims 1-24; figure 6A	22-26
X	WO 96 40968 A (UNIV LELAND STANFORD JUNIOR ;JOHN INNES CENTRE (GB)) 19 December 1996 (1996-12-19) the whole document	22-26
X	MCDANIEL R ET AL: "Multiple genetic modifications of the erythromycin polyketide synthase to produce a library of novel unnatural natural products" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,NATIONAL ACADEMY OF SCIENCE. WASHINGTON,US, vol. 96, no. 5, March 1999 (1999-03), pages 1846-1851, XP002143433 ISSN: 0027-8424 the whole document	22-26
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X	EP 0 841 402 A (NAT INST AGROBIO RES) 13 May 1998 (1998-05-13) page 3, line 28 - line 33; claim 19	44
X	US 4 963 487 A (SCHIMMEL PAUL R) 16 October 1990 (1990-10-16) column 2, line 9 - line 55	44
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 38326 A (ZINK MARY ANN ;XU GUOPING (US); HODGSON CLAGUE P (US); NATURE TECH) 3 September 1998 (1998-09-03) the whole document	
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A	YAMADA SHINYA ET AL: "Total synthesis of (-)-decarestrictine J." BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY, vol. 59, no. 9, 1995, pages 1657-1660, XP002154631 ISSN: 0916-8451 cited in the application the whole document	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	RANGANATHAN ANAND ET AL: "Knowledge-based design of bimodular and trimodular polyketide synthases based on domain and module swaps: A route to simple statin analogues." CHEMISTRY & BIOLOGY (LONDON), vol. 6, no. 10, October 1999 (1999-10), pages 731-741, XP000971117 ISSN: 1074-5521 the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/02286

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (1-8,32,33,37,38)-complete, (16-31,36,41-43, 46-48)-partially

A method of assembling several DNA units in sequence in a DNA construct, which method comprises the step of: a) providing each DNA unit with a restriction enzyme recognition sequence at its 5' end and with a recognition sequence for the same restriction enzyme at its 3' end that is combined with a restriction site for a DNA modification enzyme, b) providing a starting DNA construct having an accessible restriction site for the same or a compatible restriction enzyme and cleaving the starting DNA construct with a restriction enzyme, c) inserting the desired DNA unit and bringing the ligated product into contact with a DNA modification enzyme such that the restriction site at the 3' end of the inserted DNA unit is abolished, d) cleaving the ligated product at an accessible unmodified recognition site for the same or a compatible restriction enzyme, e) repeating step c) and d) to introduce each desired DNA unit to give a DNA construct containing all the desired units in sequence; DNA construct incorporating one or more DNA assemblies encoding synthetic enzymes and/or hosts expressing DNA constructs made by said method; compounds produced by synthetic enzymes encoded by said DNA assemblies; a method of synthesising a target molecule using said method; a method of making a synthetic enzyme to catalyse the synthesis of a target molecule using said method; a library of DNA units encoding a catalytic or transport protein domains, wherein each DNA unit has a recognition sequence for a restriction enzyme at its 5'-end and a second recognition sequence for the same or a compatible enzyme at its 3'-end which incorporates a recognition sequence for a DNA modifying enzyme; a module comprising a DNA sequence encoding a functional set of polyketide synthetic domains wherein the module has a recognition sequence for a restriction enzyme at its 5'-end and a second recognition sequence for the same or a compatible enzyme at its 3'-end which incorporates a recognition sequence for a DNA modifying enzyme; a method of transforming a host with one or more synthetic DNA assemblies encoding enzyme domains, wherein the DNA assemblies are said modules;

2. Claims: (9-15,33,34,39,40)-complete, (16-31,36,41-43, 46-48)-partially

Idem as invention 1, but limited to a method of: assembling several DNA units in sequence in a DNA construct, which method comprises the step of: a) providing a first DNA unit

with a recognition sequence for a first restriction enzyme at its 3' end, and cleaving the said first DNA unit with said first restriction enzyme, b) providing each other DNA unit with a recognition sequence at its 5' end for a second restriction enzyme which has a compatible ligation sequence with that of the first restriction enzyme, and a downstream recognition sequence for said first restriction enzyme followed by a downstream recognition sequence for a third restriction enzyme at its 3' end, and cleaving each said other DNA unit with the second and third restriction enzymes, c) ligating the said first DNA unit with a desired other DNA unit to form a ligated product such that the ligation of the two units abolishes the recognition site for the first restriction enzyme at the ligation junction, and cleaving the ligated product with said first restriction enzymel, d) ligating the product from c) with a desired DNA unit from b) to form a ligated product and cleaving the ligated product with said first restriction enzyme, e)repeating step d) with each other DNA unit in turn so as to assemble the DNA unit in sequence;

3. Claims: 44-45

A method of transforming a host with one or more synthetic DNA assemblies encoding enzyme domains which comprises the step of: a) Inserting said DNA assembly into a vector containing a mutated internal fragment of a recA gene sequence such that the vector is capable of undergoing homologous recombination with the recA gene of the host, b) bringing said vector into contact with a host chromosome under conditions which permit homologous recombination to take place, c) disrupting the host recA gene by integration of the DNA of said vector into the chromosome; said method wherein the expression vector is used to transform a Streptomyces host;

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/GB 00/02286

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