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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR GENERATING HYPERMUTABLE BACTERIA

(57) Abstract: Bacteria are manipulated to create desirable output traits using dominant negative alleles of mismatch repair proteins. Enhanced hypermutation is achieved by combination of mismatch repair deficiency and exogenously applied mutagens. Stable bacteria containing desirable output traits are obtained by restoring mismatch repair activity to the bacteria.



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INTERNATIONAL SEARCH REPORT

International Application No
 P I S 01/04339

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C12N15/10 C12N1/20

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

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)

EPO-Internal, BIOSIS, WPI Data, PAJ, SCISEARCH, CHEM ABS Data, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FISHEL R ET AL: "THE HUMAN MUTATOR GENE HOMOLOG MSH2 AND ITS ASSOCIATION WITH HEREDITARY NONPOLYPOSIS COLON CANCER" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 75, no. 5, 3 December 1993 (1993-12-03), pages 1027-1038, XP000608271 ISSN: 0092-8674 cited in the application page 1029, right-hand column, paragraph 2 -page 1030, left-hand column, paragraph 1; figure 4 page 1036, right-hand column, last paragraph -page 1037, left-hand column, paragraph 1	1,18,24, 32,36, 37,40, 41,45
Y	same citations	33-35, 38,39, 42-44, 46-50
-/--		

☒ Further documents are listed in the continuation of box C.

☐ 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

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"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

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INTERNATIONAL SEARCH REPORT

International Application No

P(S 01/04339

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	--- PRUDHOMME M ET AL: "MISMATCH REPAIR GENES OF STREPTOCOCCUS-PNEUMONIAE HEXA CONFERS A MUTATOR PHENOTYPE IN ESCHERICHIA-COLI BY NEGATIVE COMPLEMENTATION" JOURNAL OF BACTERIOLOGY, vol. 173, no. 22, 1991, pages 7196-7203, XP001013041 ISSN: 0021-9193 page 7197, left-hand column, last paragraph -right-hand column, paragraph 1 page 7199 -page 7200, left-hand column, paragraph 1; figure 1; tables 1,3	1,18,25, 32,36, 37,40, 41,45
Y	same citations	33-35, 38,39, 42-44, 46-50
X	--- ALEXANDER ARONSHTAM ET AL: "Dominant negative mutator mutations in the mutL gene of Escherichia coli" NUCLEIC ACIDS RESEARCH,OXFORD UNIVERSITY PRESS, SURREY,GB, vol. 24, no. 13, 1996, pages 2498-2504, XP002168605 ISSN: 0305-1048 cited in the application page 2499; figure 1; tables 1-4 page 2501, left-hand column -right-hand column, paragraph 1	1,18,25, 32,36, 37,40, 41,45
Y	same citations	33-35, 38,39, 42-44, 46-50
Y	--- BORNSCHEUER U T ET AL: "Directed evolution of an esterase for the stereoselective resolution of a key intermediate in the synthesis of epothilones" BIOTECHNOLOGY AND BIOENGINEERING - COMBINATORIAL CHEMISTRY,US,WILEY, NEW YORK, NY, vol. 58, no. 5, 5 June 1998 (1998-06-05), pages 554-559, XP002144278 ISSN: 0006-3592 page 556 -page 558, left-hand column, paragraph 1; figure 2 -& STRATAGENE: "XL1-Red Competent Cells" INTERNET, [Online] 2001, XP002173919 http://www.stratagene.com Retrieved from the Internet: <URL:http://www.stratagene.com/manuals/200129.pdf> [retrieved on 2001-08-02] --- -/--	33-35, 38,39, 42-44, 46-50

INTERNATIONAL SEARCH REPORT

International Application No

PI S 01/04339

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	LOH TAMALETTE ET AL: "Mutational analysis of the Muth protein from Escherichia coli." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 15, 13 April 2001 (2001-04-13), pages 12113-12119, XP002173812 ISSN: 0021-9258 page 12114, right-hand column, paragraph 2 page 12115, right-hand column, paragraphs 1-4; table 1	2
A	--- HORST JENS-PETER ET AL: "Escherichia coli mutator genes." TRENDS IN MICROBIOLOGY, vol. 7, no. 1, January 1999 (1999-01), pages 29-36, XP002173813 ISSN: 0966-842X the whole document	
A	--- FIJALKOWSKA IWONA J ET AL: "Mutants in the Exo I motif of Escherichia coli dnaQ: Defective proofreading and inviability due to error catastrophe." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 93, no. 7, 1996, pages 2856-2861, XP002173814 1996 ISSN: 0027-8424 page 2857 -page 2858, left-hand column, paragraph 1; table 1 -----	

INTERNATIONAL SEARCH REPORT

ational application No.
PCT/US 01/04339

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.:

1, 18, 24-25, 32-50

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: 1,18,24,25,32-50

A general method for making hypermutable bacteria by inducibly expressing a dominant negative allele of a mismatch repair gene in said bacteria. Furthermore a composition of cultured said hypermutable bacteria which comprise an inducible dominant negative mismatch repair gene, which is eucaryotic or procaryotic and a method for generating a mutation in a gene of interest by growing a bacterial culture or a bacterium of said inducibly hypermutable bacteria comprising said gene of interest.

2. Claim : 2

A method for making hypermutable bacteria by inducibly expressing a dominant negative allele of a mismatch repair gene in said bacteria, wherein the mismatch repair gene is MutH from any species.

3. Claims: 3,10,12,23,28,31

A method for making hypermutable bacteria by inducibly expressing a dominant negative allele of a mismatch repair gene in said bacteria, wherein the mismatch repair gene is a MutS homolog from any species or a MutS homolog from any species comprising a truncation mutation or MSH2. Furthermore a composition of cultured inducibly hypermutable bacteria, wherein the inducibly expressed dominant negative allele of said mismatch repair gene is mutS or a homolog thereof, or wherein the composition comprises a mammalian or an eucaryotic MutS protein.

4. Claims: 4,6-9,11,13-17,19-22,26,27,29,30

A method for making hypermutable bacteria by inducibly expressing a dominant negative allele of a mismatch repair gene in said bacteria, wherein the mismatch repair gene is a MutL homolog from any species or a MutL homolog from any species comprising a truncation mutation or PMS2, PMS2 comprising a truncation mutation, PMS2 comprising a truncation mutation at codon 134, plant PMS2, MLH1, MLH3, PMSR or PMSL homolog. Furthermore a composition of cultured inducibly hypermutable bacteria, wherein the inducibly expressed dominant negative allele of said mismatch repair gene is mutL, PMS2, MLH1, PMSR or a homolog thereof, or wherein the composition comprises, a protein which consists of the first 133 amino acids of PMS2 or human PMS2, a mammalian or an eucaryotic MutL protein.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claim : 5

A method for making hypermutable bacteria by inducibly expressing a dominant negative allele of a mismatch repair gene in said bacteria, wherein the mismatch repair gene is MutY from any species.

6. Claims: 51-57

A general method for enhancing the mutation rate of a bacterium by exposing a bacterium comprising a dominant negative allele of a mismatch repair gene to a mutagen as well as further secifications as to the mutagen used.

7. Claims: 58-70

A general method for generating a mismatch repair proficient bacterium with a new output trait by growing a mismatch repair deficient bacterium comprising a defective mismatch repair gene allele and a gene of interest to form a population of muated bacteria, cultivating the said mutated bacteria, testing for a muation in a gene of interest and restoring mismatch repair activtiy to at least one cultivated bacteria. Additionally further embodiments of the said method.