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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:
— *with international search report*

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: A METHOD OF ALTERING LEVELS OF PLASMIDS

(57) Abstract: The present invention relates to a method of obtaining altered plasmid contents in bacteria, bearing mutation in at least one of the chromosomal genes, nusG, rho, and dnaC, and the bacterial strains thereof, having the mutated chromosomal genes, individually or in various possible combinations, capable of altering the level of plasmids.

WO 2004/033684 A1

INTERNATIONAL SEARCH REPORT

Interr al Application No
PCT/IB 02/04500

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12N15/69 C12N15/70 C12P19/34 C12N1/21
//(C12N1/21,C12R1:19)

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)
BIOSIS, EPO-Internal, SEQUENCE SEARCH, MEDLINE, CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZELLARS MALCOLM ET AL: "Antiterminator-dependent modulation of transcription elongation rates by NusB and NusG." MOLECULAR MICROBIOLOGY, vol. 32, no. 6, June 1999 (1999-06), pages 1296-1304, XP002231298 ISSN: 0950-382X the whole document --- -/--	1,5-10, 17-23

[X] 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

"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
17 February 2003	16. 05. 2003

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	Aslund, J

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.
A	SULLIVAN S L ET AL: "REQUIREMENT FOR ESCHERICHIA-COLI NUSG PROTEIN IN FACTOR-DEPENDENT TRANSCRIPTION TERMINATION" CELL, vol. 68, no. 5, 1992, pages 989-994, XP002231299 ISSN: 0092-8674 cited in the application the whole document ---	1,5-10, 17-23
A	BUROVA ELENA ET AL: "Escherichia coli nusG mutations that block transcription termination by coliphage HK022 Nun protein." MOLECULAR MICROBIOLOGY, vol. 31, no. 6, March 1999 (1999-03), pages 1783-1793, XP002231300 ISSN: 0950-382X page 453 -page 456 ---	
A	LI JOYCE ET AL: "Elongation factor NusG interacts with termination factor rho to regulate termination and antitermination of transcription." GENES & DEVELOPMENT, vol. 7, no. 1, 1993, pages 161-172, XP008013868 ISSN: 0890-9369 ---	
A	DEL SOLAR GLORIA ET AL: "Replication and control of circular bacterial plasmids." MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, vol. 62, no. 2, June 1998 (1998-06), pages 434-464, XP002231301 ISSN: 1092-2172 ---	
A	HASUNUMA K ET AL: "EFFECT OF DNA MUTATIONS ON THE REPLICATION OF PLASMID PSC-101 IN ESCHERICHIA-COLI K-12" JOURNAL OF BACTERIOLOGY, vol. 137, no. 3, 1979, pages 1095-1099, XP008013894 EN ISSN: 0021-9193 ---	
A	BOGAN JOSEPH A ET AL: "P1 and NR1 plasmid replication during the cell cycle of Escherichia coli." PLASMID, vol. 45, no. 3, May 2001 (2001-05), pages 200-208, XP002231302 ISSN: 0147-619X figure 3 --- -/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VAN BRUNT J ET AL: "REEXAMINATION OF F PLASMID REPLICATION IN THE DNAC MUTANT OF ESCHERICHIA-COLI" MOLECULAR & GENERAL GENETICS, vol. 150, no. 3, 1977, pages 285-292, XP008013897 ISSN: 0026-8925 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 02/04500

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, 5-9, 13-16 (all partially); 2, 10, 17-23 (completely)

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:

Invention 1: Claims 1, 5-9,
13-16 (all partially); Claims 2, 10,
17-23 (completely)

A method of obtaining altered plasmid content in a bacterium bearing mutation in the chromosomal gene nusG. The specific nusG mutation altering codon 146 from encoding a Gly residue to an Asp residue. The Escherichia coli strain with accession number:NCIMB 41132 and the plasmids pHYD751 and pHYD763.

Invention 2: Claims 1, 5-9,
13-16 (all partially); Claims 3, 11,
24-25 (completely)

A method of obtaining altered plasmid content in a bacterium bearing mutation in the chromosomal gene rho. The specific rho mutation altering codon 243 from encoding an Ala residue to a Glu residue. The plasmid pHYD1201.

Invention 3: Claims 1, 5-9, 13, 15,
16 (all partially); Claims 4, 12 (completely)

A method of obtaining altered plasmid content in a bacterium bearing mutation in the chromosomal gene dnaC. The specific dnaC mutation altering codon 83 from encoding a Ala residue to a Thr residue.