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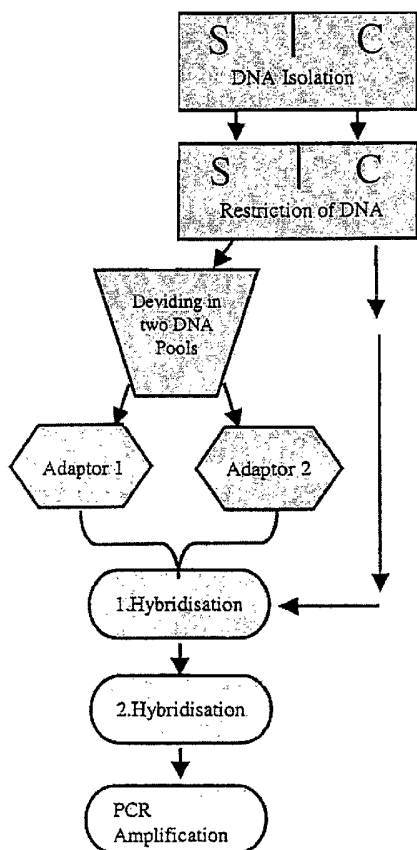
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[Continued on next page]

(54) Title: SSH BASED METHODS FOR IDENTIFYING AND ISOLATING UNIQUE NUCLEIC ACID SEQUENCES



(57) Abstract: A subtractive suppression hybridization (SSH) assay and uses thereof are described. In particular, methods of identifying and isolating nucleic acid sequences, which are unique for a certain cell, tissue or organism are provided, wherein said unique nucleic acid sequences are related to for example diseases genes. More specifically, SSH assays for unique genomic DNA sequences and improved SSH assays that are combined with 2D gel electrophoresis techniques are provided. The presented methods are particular useful for the identification of genes involved in the development of various diseases, including cancer, hypertension and diabetes as well as for monitoring animals and food, for example for infection agents and other contaminants.

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European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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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.

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/EP 03/04570

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12N15/10 G01N33/50

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 C12Q C12N G01N

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, MEDLINE, WPI Data, PAJ, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 49345 A (SMITH CASSANDRA L ;UNIV BOSTON (US)) 5 November 1998 (1998-11-05) abstract; figure 4 ---	1-9, 11-22, 24-30
A	WO 96 23079 A (CLONTECH LAB INC) 1 August 1996 (1996-08-01) cited in the application abstract; figure 4 --- -/--	1-9, 11-22, 24-30

☒ 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

15 October 2003

Date of mailing of the international search report

06. 11. 03

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/04570

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DIATCHENKO L ET AL: "SUPPRESSION SUBTRACTIVE HYBRIDIZATION: A METHOD FOR GENERATING DIFFERENTIALLY REGULATED OR TISSUE-SPECIFIC CDNA PROBES AND LIBRARIES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, 1 June 1996 (1996-06-01), pages 6025-6030, XP002911922 ISSN: 0027-8424 cited in the application abstract; figure 1 ----	1-9, 11-22, 24-30
A	JANKE B ET AL: "A subtractive hybridisation analysis of genomic differences between the uropathogenic E. coli strain 536 and the E. coli K-12 strain MG1655." FEMS MICROBIOLOGY LETTERS. NETHERLANDS 15 MAY 2001, vol. 199, no. 1, 15 May 2001 (2001-05-15), pages 61-66, XP002249024 ISSN: 0378-1097	1-9, 11-22, 24-30
Y	abstract page 62, column 1, paragraph 2.3 page 62, column 2, paragraph 3.1 ----	10
X	TASHIMA L S ET AL: "Genes upregulated in human fetal membranes by infection or labor." OBSTETRICS AND GYNECOLOGY. UNITED STATES SEP 1999, vol. 94, no. 3, September 1999 (1999-09), pages 441-449, XP001154999 ISSN: 0029-7844	11-13, 18, 20-25, 27-30
Y	abstract page 4429, column 2, paragraph 2; figure 1 ----	10
X,P	MOHAN M ET AL: "Analysis of gene expression in the bovine blastocyst produced in vitro using suppression-subtractive hybridization." BIOLOGY OF REPRODUCTION. UNITED STATES AUG 2002, vol. 67, no. 2, August 2002 (2002-08), pages 447-453, XP009017791 ISSN: 0006-3363 abstract ----- -/--	23-25, 27-30

INTERNATIONAL SEARCH REPORT

Internati lication No
PCT/EP 03/04570

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HARMS C ET AL: "Two-dimensional agarose gel electrophoresis as a tool to isolate genus- and species-specific repetitive DNA sequences." ANALYTICAL BIOCHEMISTRY. UNITED STATES 15 AUG 2000, vol. 284, no. 1, 15 August 2000 (2000-08-15), pages 6-10, XP002257000 ISSN: 0003-2697 the whole document -----	21,22
X,P	HARMS C ET AL: "Characterization of minute differences between genomes of strains of <i>Penicillium nalgiovense</i> using subtractive suppression hybridization without cloning." LETTERS IN APPLIED MICROBIOLOGY. ENGLAND 2002, vol. 35, no. 2, 2002, pages 113-116, XP002256998 ISSN: 0266-8254 the whole document -----	1-30

INTERNATIONAL SEARCH REPORT

Inter application No.
PCT/EP 03/04570

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:

Although claim 28 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
- 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: 1-9, 11-13(partly), 14-17, 18(partly), 19, 20-22(partly), 24-25(partly), 26, 27(partly) 28, 29-30(partly)

A method of identifying and/or isolating a unique nucleic acid fragment or/and a gene encoded thereby according to steps (a) - (f) of present claim 1, and in accordance with all the above claims which are dependent on claim 1, with the provision that the tester nucleic acid sample comprises or consists of eukaryotic genomic DNA fragments. A method of diagnosis, a kit and the use of the method according to claims 28, 29 and 30 in relation to the above subejct matter.

2. Claims: 10, 11-13(partly), 18(partly), 20-22(partly), 23, 24-25(partly), 27(partly), 29-30(partly)

A method of identifying and/or isolating a unique nucleic acid fragment or/and a gene encoded thereby according to steps (a) - (f) of present claim 1, and in accordance with all the above claims which are dependent on claim 1, with two provision that, first, the driver nucleic acid sample comprises a pool of nucleic acids and, second, the tester nucleic acid sample comprises or consists of cDNA, fragments thereof or prokaryotic or viral DNA or fragments thereof. A kit and the use of the method according to claims 29 and 30 in relation to the above subejct matter.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP 03/04570

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WO 9849345	A	05-11-1998	AU 7265298 A WO 9849345 A1	24-11-1998 05-11-1998
WO 9623079	A	01-08-1996	US 5565340 A AU 4774196 A EP 0753075 A1 JP 9511149 T WO 9623079 A2 US 5759822 A	15-10-1996 14-08-1996 15-01-1997 11-11-1997 01-08-1996 02-06-1998