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(54) Title: DETECTION OF EXTRACELLULAR TUMOR-ASSOCIATED NUCLEIC ACID IN BLOOD PLASMA OR SERUM

(57) Abstract: This invention relates to detection of specific extracellular nucleic acid in plasma or serum fractions of human or animal blood associated with neoplastic, pre-malignant or proliferative disease. Specifically, the invention relates to detection of nucleic acid derived from mutant oncogenes or other tumor-associated DNA, and to those methods of detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with or without enrichment for mutant DNA.

INTERNATIONAL SEARCH REPORT

Inte | Application No
PCT/US 00/32587

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12P19/34 C07H21/04 C07K1/00

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

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, 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.
X	WO 97 34015 A (PENN STATE RES FOUND) 18 September 1997 (1997-09-18)	1-6, 10-20, 24-29, 33-39, 50-68
Y	* whole document *	7-9, 21-23, 30-32, 40-49
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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	"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
"E" earlier document but published on or after the international filing date	"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
"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)	"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.
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 7 June 2002	Date of mailing of the international search report 24 06 2002
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Name and mailing address of the ISA 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	Authorized officer Aguilera, M
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INTERNATIONAL SEARCH REPORT

 Inte: Application No
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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.
X	WO 96 15139 A (AMERICAN HEALTH FOUND ;RONAI ZEEV (US)) 23 May 1996 (1996-05-23)	1-6, 10-20, 24-29, 33-39, 50-68
Y	* whole document *	7-9, 21-23, 30-32, 40-49
X	--- SORENSEN G D ET AL: "Soluble normal and mutated DNA sequences from single-copy genes in human blood." CANCER EPIDEMIOLOGY, BIOMARKERS AND PREVENTION, (1994 JAN-FEB) 3 (1) 67-71., XP001064067 cited in the application	1-6, 10-20, 24-29, 33-39, 50-68
Y	* whole document *	7-9, 21-23, 30-32, 40-49
Y	--- US 5 496 699 A (SORENSEN GEORGE D) 5 March 1996 (1996-03-05) column 1, line 10 -column 9, line 15; figures 1A,1B	1-68
Y	--- WO 95 16792 A (STROUN MAURICE ;ANKER PHILIPPE (CH); VASIOUKHIN VALERI (US)) 22 June 1995 (1995-06-22) example 1	1-68
Y	--- KAHN S M ET AL: "RAPID AND SENSITIVE NONRADIOACTIVE DETECTION OF MUTANT K-RAS GENES VIA ENRICHED PCR AMPLIFICATION" ONCOGENE, vol. 6, no. 6, 1991, pages 1079-1084, XP002191761 ISSN: 0950-9232 cited in the application * whole document *	1-68
Y	--- SIDRANSKY D ET AL: "Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 256, 3 April 1992 (1992-04-03), pages 102-105, XP000605488 ISSN: 0036-8075 * Abstract * page 103, column 2, paragraph 2 -column 3, paragraph 2 --- -/--	1-68

INTERNATIONAL SEARCH REPORT

Int Application No
PCT/US 00/32587

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZEHNDER J ET AL: "CROSS-LINKING HYBRIDIZATION ASSAY FOR DIRECT DETECTION OF FACTOR V LEIDEN MUTATION" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY, WINSTON, US, vol. 43, no. 9, September 1997 (1997-09), pages 1703-1708, XP002928152 ISSN: 0009-9147 * Materials and Methods *	7-9, 21-23, 30-32, 40-49
Y	WO 98 58081 A (EXACT LAB INC) 23 December 1998 (1998-12-23) page 5, line 12 -page 7, line 11; example 4	7-9, 21-23, 30-32, 40-49
Y	HORNES E ET AL: "MAGNETIC DNA HYBRIDIZATION PROPERTIES OF OLIGONUCLEOTIDE PROBES ATTACHED TO SUPERPARAMAGNETIC BEADS AND THEIR USE IN THE ISOLATION OF POLY(A) MRNA FROM EUKARYOTIC CELLS" GENETIC ANALYSIS, ELSEVIER, AMSTERDAM, NL, vol. 7, no. 6, 1 October 1990 (1990-10-01), pages 145-150, XP000198013 ISSN: 1050-3862 * whole document *	7-9, 21-23, 30-32, 40-49
Y	LANKIEWICZ SILKE ET AL: "Enhanced RACE method using specific enrichment by biotinylated oligonucleotides bound to streptavidin coated magnetic particles." NUCLEIC ACIDS RESEARCH, vol. 25, no. 10, 1997, pages 2037-2038, XP002201590 ISSN: 0305-1048 * whole document *	7-9, 21-23, 30-32, 40-49
Y	ALBRETSSEN C ET AL: "APPLICATIONS OF MAGNETIC BEADS WITH COVALENTLY ATTACHED OLIGONUCLEOTIDES IN HYBRIDIZATION ISOLATION AND DETECTION OF SPECIFIC MEASLES VIRUS MESSENGER RNA FROM A CRUDE CELL LYSATE" ANALYTICAL BIOCHEMISTRY, vol. 189, no. 1, 1990, pages 40-50, XP008004133 ISSN: 0003-2697 * whole document *	7-9, 21-23, 30-32, 40-49

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 00/32587

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>VAN MANSFELD A.D.M. ET AL.: "PCR-based Approaches for Detection of Mutated ras Genes" PCR METHODS AND APPLICATIONS, vol. 1, pages 211-216, XP001064082 Cold Spring Harbor Lab. Press * whole document *</p> <p style="text-align: center;">-----</p>	1-68

INTERNATIONAL SEARCH REPORT

International application No.
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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: claims 1, 11, 25 (all partially); 2-6, 10, 12-20, 24, 26-29, 33-39, and 50-68 (all completely)

Methods for the detection of oncogenic extracellular DNA from plasma or serum comprising the steps of: (a) purification of extracellular nucleic acid from blood; (b) optionally, enrichment for mutant DNA using selective restriction enzyme digestion; (c) amplification and (d) detection of said mutant oncogene.

2. Claims: 1, 11, 25 (all partially); 7-9, 21-23, 30-32, and 40-49 (all completely)

Methods for the detection of oncogenic extracellular DNA from plasma or serum comprising the steps of: (a) purification of extracellular nucleic acid from blood; (b) enrichment for mutant DNA using oligonucleotide probe hybridization; (c) amplification and (d) detection of said mutant oncogene.

INTERNATIONAL SEARCH REPORT

onal Application No
PCT/US 00/32587

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