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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : **G01N 33/68**	**A3**	**(11) International Publication Number:** **WO 99/12040** **(43) International Publication Date:** 11 March 1999 (11.03.99)

(21) International Application Number: PCT/US98/18311

(22) International Filing Date: 2 September 1998 (02.09.98)

(30) Priority Data:
08/922,201 2 September 1997 (02.09.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
2 September 1999 (02.09.99)

(54) Title: MASS SPECTROMETRIC DETECTION OF POLYPEPTIDES

(57) Abstract

A process for determining the identity of a target polypeptide using mass spectroscopy is provided. Depending on the target polypeptide to be identified, a process as disclosed can be used, for example, to diagnose a genetic disease or chromosomal abnormality, a predisposition to a disease or condition, or infection by a pathogenic organism; or for determining identity or heredity. Kits for performing the disclosed processes also are provided.

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INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 98/18311

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68

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 6 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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 11249 A (GARVIN ALEX M) 19 March 1998 — see page 4, paragraph 5 - page 5, paragraph 2 see page 6, line 4 - line 9 --- -/--	1-11, 17-28, 34, 38-44, 48,49, 52,55, 58-60, 63,64, 76,107, 108,111

[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)

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"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
11 June 1999	14. 07. 99

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	Hart-Davis, J

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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	US 5 538 897 A (YATES III JOHN R ET AL) 23 July 1996 see column 17, line 26 - column 18, line 56; claim 1	2,28,29, 31,34, 37-44, 48,49, 52,55, 58,60, 63-65, 71,72, 74,76
X	PROME D ET AL: "Use of Combined Mass Spectrometry Methods for the Characterization of a New Variant of Human Hemoglobin: The Double Mutant Hemoglobin Villeparisis beta77(EF1) His @rarr Tyr, beta80 (EF4) Asn @rarr Ser" JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, vol. 7, no. 2, February 1996, page 163-167 XP004051911 see the whole document	2,28, 39-44, 49,52, 55,58, 71,72,74 78, 80-83, 85,86, 89,93, 97,98, 100,101, 104,105, 113,114, 117-119
X	A MOSCA, R PALEARI, F M RUBINO, L ZECCA, G DE BELLIS, S DEBERNADI, F BAUDO, D CAPPELLINI, G FIORELLI: "Hb Abrruzzo '.beta.143(H21)His>-Arg! Identified by Mass Spectrometry and DNA Analysis" HEMOGLOBIN, vol. 17, no. 3, 1993, pages 261-268, XP002093188 see the whole document	2,28, 39-44, 49,52, 55,58, 71,72,74 78, 80-83, 85,86, 89,93, 97,98, 100,101, 104,105, 113,114, 117-119

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INTERNATIONAL SEARCH REPORT

Int itional Application No
PCT/US 98/18311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X A	GB 2 168 478 A (SCAN LIMITED M) 18 June 1986 see the whole document	2,28,39, 40,58 78, 80-86, 89,93, 97-101, 104,105, 113,114, 117-119

X X	WO 96 36732 A (UNIV ROCKEFELLER ;SCRIPPS RESEARCH INST (US); CIPHERGEN BIOSYSTEMS) 21 November 1996 cited in the application see page 5, line 32 - page 7, line 38	2,28,39, 40,58 58,78, 80-83, 85,86, 89,93, 97,98, 100,101, 104,105, 113,114, 117-119

A	WO 95 31429 A (UNIV BOSTON) 23 November 1995 cited in the application see page 29; figure 7; table 4 & US 5 643 722 A (ROTHSCHILD KENNETH J ET AL) 1 July 1997 cited in the application	2,34-36, 58,60-62

X X	WO 93 24834 A (BEAVIS RONALD ;CHAIT BRIAN T (US); WANG RONG (US); KENT STEPHEN B) 9 December 1993 cited in the application see page 30; claims 1-10; figures 3,4	2,28,39, 40,58 78, 80-84, 89,93, 97-99, 104,105, 113,114, 117,118

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PCT/US 98/18311

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 94 28418 A (BAYLOR COLLEGE MEDICINE ;HUTCHENS T WILLIAM (US); YIP TAI TUNG (US) 8 December 1994	2,14-16, 28-40, 58,60-65
X	see page 16, line 10 - page 17, line 21 see page 59, line 20 - page 60, line 25 see page 67, line 18 - page 68, line 11 see page 81, line 1 - page 84, line 6 see page 29, line 9 - page 33, line 10	78, 80-83, 85-98, 100-106, 113-119

X	WO 97 19110 A (STRATTON MICHAEL RUDOLF ;WOOSTER RICHARD FRANCIS (GB); ASHWORTH AL) 29 May 1997	2,28,39, 40,58
A	see page 48, line 25 - line 33; examples 3,4	41-44, 48,49, 52,55, 71,72, 74,76

X	EP 0 683 234 A (TAKEDA CHEMICAL INDUSTRIES LTD) 22 November 1995	2,28,39, 40,58
A	see page 16, line 22 - line 36	29,31, 34,37, 38, 41-44, 49,52, 55,60, 63-65, 71,72,74

X	WO 96 36986 A (PERSEPTIVE BIOSYSTEMS INC) 21 November 1996	2,28,39, 40,58
X	see claims 1,12-17	58,78, 80-83, 85,86, 89,93, 97,98, 100,101, 104,105, 113,114, 117-119

X	WO 95 25737 A (PENN STATE RES FOUND ;BENKOVIC STEPHEN J (US); WINOGRAD NICHOLAS ( ) 28 September 1995 see page 28, line 12 - page 30, line 25; examples 4,5	2,28, 34-36, 38-40, 58,60-64

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INTERNATIONAL SEARCH REPORT

Int :tional Application No
PCT/US 98/18311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	A BELANGER, H VAN HALBEEK, H C GRAVES, K GRANDBOIS, T A STAMEY, L HUANG, I POPPE, F LABRIE: "Molecular mass and carbohydrate structure of prostate specific antigen: studies for establishment of an international PSA standard" PROSTATE, vol. 27, no. 4, October 1995, pages 187-197, XP002105696 see the whole document ---	2,28, 39-47, 49,52, 55,58, 71,72, 74,75
X	A C TAS, J ODINK, J VAN DER GREEF, M D FERRARI, L VAN EKDOM, A C PETERS, W BOOGERD: "Characterization of virus infected cell cultures by pyrolysis / direct chemical ionization mass spectrometry" BIOMEDICAL AND ENVIRONMENTAL MASS SPECTROMETRY, vol. 18, no. 9, September 1989, pages 757-760, XP002105697 see the whole document ---	2,28,39, 40,56-58
P,X	A M HAAG, S N TAYLOR, K H JOHNSTON, R B COLE: "Rapid identification and speciation of Haemophilus bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry" JOURNAL OF MASS SPECTROMETRY, vol. 33, no. 8, 1998, pages 750-756, XP002105698 see the whole document ---	2,28,39, 40,56-58
P,X	K J WELHAM, M A DOMIN, D E SCANNELL, E COHEN, S ASHSTON: "The rapid identification of intact microorganisms by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry" PHARMACY AND PHARMACOLOGY COMMUNICATIONS, vol. 4, no. 2, February 1998, pages 81-87, XP002105699 see the whole document ---	2,28,39, 40,56-58
X	T KRISHNAMURTHY, P L ROSS, M T GOODE, D L MENKING, U RAJAMANI: "Biomolecules and mass spectroscopy" JOURNAL OF NATURAL TOXINS, vol. 6, no. 2, 1997, pages 121-162, XP002105700 see the whole document --- -/--	2,28,39, 40,56-58

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INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 98/18311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	T KRISHNAMURTHY, P L ROSS: "Rapid identification of bacteria by direct matrix assisted laser desorption / ionization mass spectrometric analysis of whole cells" RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 10, no. 15, 1996, pages 1992-1996, XP002105701 see the whole document	2,28,39, 40,56-58

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

INTERNATIONAL SEARCH REPORT

Ir. .national application No.
PCT/US 98/18311

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.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98/18311

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, 2 (in part), 3-13, 14-16 (in part), 17-27, 28-38 (in part), 39-40, 41-58 (in part), 59, 66-70

   Use of mass spectroscopy in the identification of polypeptides, the polypeptides having been obtained by prior translation of nucleic acids. Claims 14-16 and 28-57 have been searched in so far as they as they refer back to Claim 1 (these claims as filed depend on Claims 1 or 2).

2. Claims: 2 (in part), 14-16 (in part), 30-33 (in part)

   Use of mass spectroscopy in the identification of polypeptides, the polypeptide comprising a tag moiety. Claims 14-16 and 30-33 have been searched in so far as they as they refer back to Claim 2 (these claims as filed depend on Claims 1 or 2).

3. Claims: 2 (in part), 28-29 (in part), 34-38 (in part), 58 (in part), 60-65

   Use of mass spectroscopy in the identification of a polypeptide or of a plurality of polypeptides, the polypeptide(s) having been immobilized on a solid support prior to analysis by mass spectroscopy. Claims 34-38 have been searched in so far as they as they refer back to Claim 2 (these claims as filed depend on Claims 1 or 2).

4. Claims: 2 (in part), 41-57 (in part), 71-77

   Use of mass spectroscopy in the identification of polypeptides, the polypeptide(s) being associated with allelic variants and/or disease states. Claims 41-57 have been searched in so far as they as they refer back to Claim 2 (these claims as filed depend on Claims 1 or 2).

5. Claims: 78-120

   Use of mass spectroscopy in the identification of polypeptides, the polypeptide(s) having been treated prior to MS analysis by an agent which cleaves peptide bonds in the said polypeptides, thus producing peptide fragments.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 98/18311

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9811249 A	19-03-1998	NONE	
US 5538897 A	23-07-1996	CA 2185574 A	21-09-1995
		EP 0750747 A	02-01-1997
		JP 9510780 T	28-10-1997
		WO 9525281 A	21-09-1995
GB 2168478 A	18-06-1986	US 4701419 A	20-10-1987
WO 9636732 A	21-11-1996	US 5792664 A	11-08-1998
		AU 5749096 A	29-11-1996
		CA 2221727 A	21-11-1996
		EP 0883692 A	16-12-1998
WO 9531429 A	23-11-1995	US 5643722 A	01-07-1997
		AU 2635995 A	05-12-1995
		CA 2189848 A	23-11-1995
		EP 0763009 A	19-03-1997
		JP 10500409 T	13-01-1998
WO 9324834 A	09-12-1993	AU 685055 B	15-01-1998
		AU 4395893 A	30-12-1993
		CA 2136717 A	09-12-1993
		EP 0664884 A	02-08-1995
		JP 7507394 T	10-08-1995
		US 5792664 A	11-08-1998
WO 9428418 A	08-12-1994	AU 676582 B	13-03-1997
		AU 7048394 A	20-12-1994
		CA 2163426 A	08-12-1994
		EP 0700521 A	13-03-1996
		JP 9501489 T	10-02-1997
		NZ 267842 A	22-09-1997
		US 5719060 A	17-02-1998
		US 5894063 A	13-04-1999
WO 9719110 A	29-05-1997	AU 7635096 A	11-06-1997
		CA 2238010 A	29-05-1997
		EP 0858467 A	19-08-1998
		GB 2307477 A,B	28-05-1997
EP 0683234 A	22-11-1995	WO 9417197 A	04-08-1994
		US 5750349 A	12-05-1998
WO 9636986 A	21-11-1996	US 5869240 A	09-02-1999
		EP 0827628 A	11-03-1998
		US 5827659 A	27-10-1998
		US 5821063 A	13-10-1998
WO 9525737 A	28-09-1995	EP 0751950 A	08-01-1997
		JP 9510711 T	28-10-1997
		US 5834195 A	10-11-1998