# (19) World Intellectual Property Organization International Bureau



## 

(43) International Publication Date 3 August 2000 (03.08.2000)

**PCT** 

# (10) International Publication Number WO 00/44935 A3

(51) International Patent Classification7: C12Q 1/68, 1/70

(21) International Application Number: PCT/EP00/00677

(22) International Filing Date: 28 January 2000 (28.01.2000)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PA 1999 00114

29 January 1999 (29.01.1999) DF

(71) Applicant (for all designated States except US): BAVARIAN NORDIC RESEARCH INSTITUTE A/S [DK/DK]; Vesterbrogade 149, DK-1620 Copenhagen V (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KLEIN, Dieter [DE/AT]; Mollersdorf 50, A-3430 Tulln (AT). GÜNZBURG, Walter [AT/AT]; Weyprechtgasse 10, A-2340 Mödling (AT). SALMONS, Brian [GB/DE]; Mitterfeldstrasse 11, D-85229 Ainhofen (DE).

(74) Agent: PIELKEN, Petra; Bavarian Nordic Research Institute GmbH, Fraunhoferstrasse 18b, D-82152 Martinsried (DE).

(81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, 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, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, 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

(88) Date of publication of the international search report:

4 October 2001

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: MULTIPLEX REAL-TIME PCR

(57) Abstract: The present invention relates to a real-time Polymerase Chain Reaction (PCR) method for the detection and quantification of variants of nucleic acid sequences which differ in the probe-bindung site. The method is based on the complete or partial amplification of the same region of the variants and the addition of two or more oligonucleotide probes to the same PCR mixture, each probe being specific for the probe-binding site of at least one variant. The method can be applied e.g. to estimate the viral load in a sample, to differentiate between subgroups, subtypes isolates or clades of a viral species or to estimate the impact of the viral load on tumorigenesis.

## INTERNATIONAL SEARCH REPORT Intel Shall Application No

Intel onal Application No PCT/FP 00/00677

		PCT/EP 00,	/00677
A. CLASSIF IPC 7	FICATION OF SUBJECT MATTER C12Q1/68 C12Q1/70		
According to	International Patent Classification (IPC) or to both national classifica	tion and IPC	
	SEARCHED		
Minimum do	cumentation searched (classification system followed by classificatio C12Q	n symbols)	
	ion searched other than minimum documentation to the extent that su		
1	ata base consulted during the international search (name of data bas ternal, STRAND, WPI Data, PAJ, MEDLI		
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the rele	evant passages	Relevant to claim No.
X	LETTENEGGER C ET AL: "Rapid feli immunodeficiency virus provirus quantitation by polymerase chain using the TAQMAN.RTM. fluorogenic real-time detection system" JOURNAL OF VIROLOGICAL METHODS,NL,AMSTERDAM, vol. 78, no. 78, January 1999 (19 pages 105-116, XP002103560 ISSN: 0166-0934 cited in the application the whole document	reaction	1-9,12, 16-21, 23-25, 29,30
X Fur	ther documents are listed in the continuation of box C.	Patent family members are listed	in annex.
"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		"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	e actual completion of the international search	Date of mailing of the international se	arch report
2	24 August 2000	19/09/2000	
Name and	mailing address of the ISA  European Patent Office, P.B. 5818 Patentlaan 2  NL – 2280 HV Rijswijk	Authorized officer	
	Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Knehr, M	

1

### INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/EP 00/00677

0.40	PCT/EF UU/UUD//	
	ation) DOCUMENTS CONSIDERED TO BE RELEVANT  Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Category °	onation of quotinent, with indication, where appropriate, of the relevant passages	ricievani io Galin No.
A	HEID C A ET AL: "REAL TIME QUANTITATIVE PCR" GENOME RESEARCH,US,COLD SPRING HARBOR LABORATORY PRESS, vol. 6, no. 10, 1 October 1996 (1996-10-01), pages 986-994, XP000642795 ISSN: 1088-9051 cited in the application	
	the whole document	
Α	VAHLENKAMP T W ET AL: "COMPETITIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION FOR QUANTITATION OF FELINE IMMUNODEFICIENCY VIRUS" JOURNAL OF VIROLOGICAL METHODS,NL,AMSTERDAM, vol. 52, no. 3, 1 April 1995 (1995-04-01), pages 335-346, XP000609892 ISSN: 0166-0934 the whole document	
Α	GERARD ET AL: "IMPROVED QUANTITATION OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA USING REAL-TIME POLYMERASE CHAIN REACTION AND PLASMID-DNA COMPLEMENTARITY DETERMINING REGION III STANDARDS" CANCER RESEARCH, US, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 58, 1 September 1998 (1998-09-01), pages 3957-3964, XP002095189 ISSN: 0008-5472 the whole document	
Α	WO 96 40268 A (AMERICAN HOME PROD) 19 December 1996 (1996-12-19) abstract page 15, line 14	
P,X	KLEIN D ET AL.: "Proviral load determination of different feline immunodeficiency virus isolates using real-time polymerase chain reaction: Influence of mismatches on quantification" ELECTROPHORESIS, vol. 20, 1999, pages 291-299, XP000924950 the whole document	1-9,12, 16-25, 29,30

1

## INTERNATIONAL SEARCH REPORT

Inter anal Application No
PCT/EP 00/00677

0.40	New DOCUMENTS CONCIDENTS TO BE SELECTION.	101/21 00/000//
	tion) DOCUMENTS CONSIDERED TO BE RELEVANT  Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	VET J A M ET AL.: "Multiplex detection of four pathogenic retroviruses using molecular beacons" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, vol. 96, 1999, pages 6394-6399, XP002145609 the whole document	Relevant to claim No.  1-5,7, 18-22, 29,30

1

### INTERNATIONAL SEARCH REPORT

information on patent family members

Inter onal Application No
PCT/EP 00/00677

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9640268 A	19-12-1996	US 5820869 A	13-10-1998
		AU 699794 B	17-12-1998
		AU 5973196 A	30-12-1996
		BR 9609089 A	02-02-1999
		CA 2224257 A	19-12-1996
		EP 0831921 A	01-04-1998
		JP 11506614 T	15-06-1999
		US 5989562 A	23-11-1999