

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
13 March 2003 (13.03.2003)

PCT

(10) International Publication Number  
WO 03/020931 A3

(51) International Patent Classification<sup>7</sup>: C12N 15/11,  
5/10, 7/00, A61K 31/713

(21) International Application Number: PCT/EP02/09670

(22) International Filing Date: 29 August 2002 (29.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/317,229 1 September 2001 (01.09.2001) US  
60/385,733 4 June 2002 (04.06.2002) US

(71) Applicant (for all designated States except US): GALA-  
PAGOS GENOMICS N.V. [BE/BE]; Generaal de Witte-  
laan 11 A3, B-2800 Mechelen (BE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): ARTS, Gert-Jan

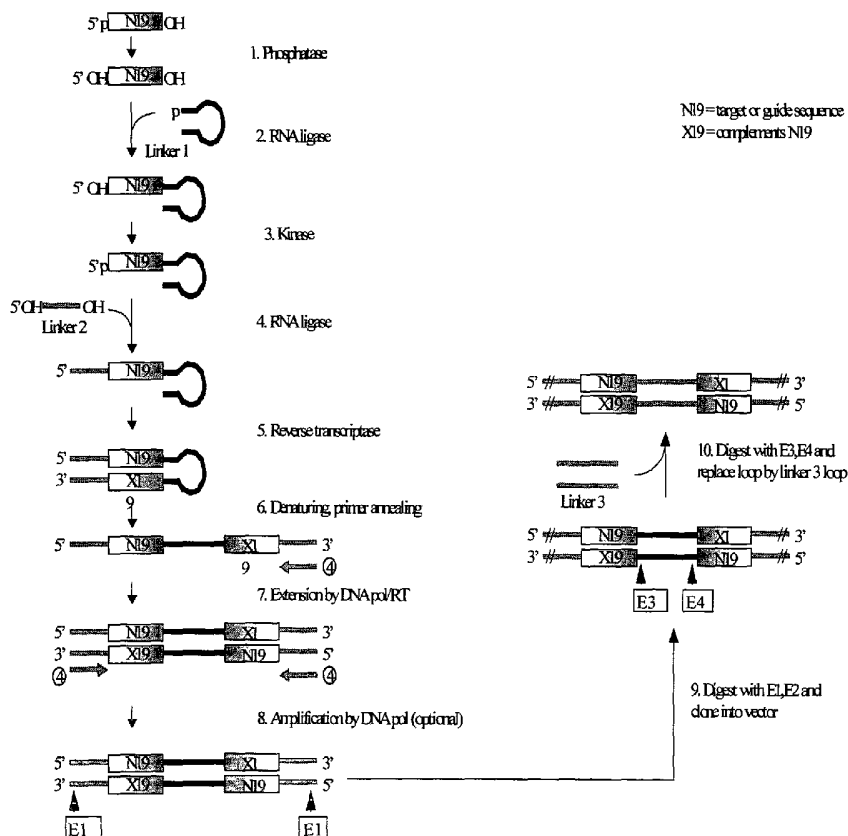
[NL/NL]; Wikkestraat 46, NL-2403 ES Alphen AD Rijn (NL). LANGEMEIJER, Ellen, Vera [NL/NL]; Simonsstraat 87, NL-2628 TG Delft (NL). PIEST, Ivo [NL/NL]; Waverbancken 16, NL-3645 VS Vinkeveen (NL). VAN ES, Helmut, Hendrikus, Gerardus [NL/NL]; Breesstraat 18, NL-2011 ZZ Haarlem (NL). MICHIELS, Godefridus, Augustinus, Maria [NL/NL]; Vronkenlaan 12, NL-2352 EP Leiderdorp (NL).

(74) Agent: HOOIVELD, Arjen, J., W.; Arnold & Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL).

(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, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: SIRNA KNOCKOUT ASSAY METHOD AND CONSTRUCTS



(57) Abstract: The present invention relates to polynucleotide constructs, methods for their preparation, and preparations for their use in methods that lower the amount of RNA and/or protein production in cells based on the intracellular expression of small interfering polyribonucleic acid molecules.



WO 03/020931 A3



**(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).

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

**(88) Date of publication of the international search report:**  
20 November 2003

**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.*

## INTERNATIONAL SEARCH REPORT

 International Application No  
 PCT/EP 02/09670

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 7 C12N15/11 C12N5/10 C12N7/00 A61K31/713		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N A61K		
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, BIOSIS, EMBASE		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUTVAGNER GYORGY ET AL: "A cellular function for the RNA-interference enzyme dicer in the maturation of the let-7 small temporal RNA." SCIENCE (WASHINGTON D C), vol. 293, no. 5531, 3 August 2001 (2001-08-03), pages 834-838, XP002204649 ISSN: 0036-8075	1-4, 10-13, 21,32
Y	page 838, middle column, line 4 - last line ; figure 5	17,20, 23-29
Y	WO 99 64582 A (INTROGENE BV) 16 December 1999 (1999-12-16) cited in the application examples 3,9,16,20 claims	17,20, 23-29
	----- -/--	
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> 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 3 September 2003		Date of mailing of the international search report 22/09/2003
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 Andres, S.

## INTERNATIONAL SEARCH REPORT

 International Application No  
 PCT/EP 02/09670

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 00 49035 A (GEN HOSPITAL CORP) 24 August 2000 (2000-08-24)  page 11, line 15 - page 14, line 14 claims; figure 1  -----	1-4, 10-13, 17,21, 22,29,32
X	PASQUINELLI AMY E ET AL: "Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA." NATURE (LONDON), vol. 408, no. 6808, 2000, pages 86-89, XP002204652. ISSN: 0028-0836 cited in the application the whole document  -----	1-3,10, 21
X	WO 96 29097 A (RES CORP TECHNOLOGIES INC) 26 September 1996 (1996-09-26)  page 6 - page 8 page 26, line 6 - line 20 page 52 claims  -----	1,2,4, 17,21, 23,29,32
A	ELBASHIR SAYDA M ET AL: "RNA interference is mediated by 21- and 22-nucleotide RNAs." GENES & DEVELOPMENT, vol. 15, no. 2, 15 January 2001 (2001-01-15), pages 188-200, XP002204651 ISSN: 0890-9369  -----	
A	MEDINA M F C ET AL: "RNA POLYMERASE III-DRIVEN EXPRESSION CASSETTES IN HUMAN GENE THERAPY" CURRENT OPINION IN MOLECULAR THERAPEUTICS, vol. 1, no. 5, October 1999 (1999-10), pages 580-594, XP001055319 ISSN: 1464-8431 the whole document  -----	17-20, 23,25-28
A	WO 00 52188 A (ELLARD FIONA MARGARET ; KINGSMAN SUSAN MARY (GB); MITROPHANOUS KYRIACO) 8 September 2000 (2000-09-08) page 8 page 22 - page 26 claims; examples  -----	30,31
A	WO 00 11154 A (DONAHUE BRIAN A ; HARDY STEPHEN F (US); CELL GENESYS INC (US); SNYDER) 2 March 2000 (2000-03-02) page 16 - page 18 claims  -----	30,31

-/--

## INTERNATIONAL SEARCH REPORT

Int. Patent Application No  
PCT/EP 02/09670

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>PADDISON PATRICK J ET AL: "Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells." GENES &amp; DEVELOPMENT, vol. 16, no. 8, 15 April 2002 (2002-04-15), pages 948-958, XP002204653 ISSN: 0890-9369 the whole document</p>	1-4, 10-13, 17-19, 21-24, 29,32
P, X	<p>YU JENN-YAH ET AL: "RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 99, no. 9, 30 April 2002 (2002-04-30), pages 6047-6052, XP002204654 ISSN: 0027-8424 the whole document</p>	1,2,10, 17,19, 21,23, 29,32
P, X	<p>RAYKOV Z ET AL: "Transient suppression of transgene expression by means of antisense oligonucleotides: A method for the production of toxin-transducing recombinant viruses." GENE THERAPY, vol. 9, no. 5, March 2002 (2002-03), pages 358-362, XP002253225 ISSN: 0969-7128 the whole document</p>	30,31
E	<p>WO 03 012082 A (ANDREWS PETER ; AXORDIA LTD (GB); WALSH JAMES (GB); GOKHALE PAUL (GB)) 13 February 2003 (2003-02-13) page 7, line 23 - page 8 page 12, line 29 - page 13, line 2 page 23, line 24 - page 25, line 13 claims figure 3 on page 3/41 of the figures</p>	1-4,10, 11,17, 21,29,32

# INTERNATIONAL SEARCH REPORT

national application No.  
PCT/EP 02/09670

## 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 claims 21 (as far as in vivo methods are concerned) and 32 are directed to a method of treatment of 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:

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

Information on patent family members

International Application No

PCT/EP 02/09670

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9964582	A	16-12-1999	US 6413776 B1	02-07-2002
			AU 756605 B2	16-01-2003
			AU 4294799 A	30-12-1999
			CA 2301403 A1	16-12-1999
			EP 1022335 A1	26-07-2000
			JP 2002526031 T	20-08-2002
			WO 9964582 A2	16-12-1999
			US 2003027170 A1	06-02-2003
			US 2003059794 A1	27-03-2003
			US 6340595 B1	22-01-2002
WO 0049035	A	24-08-2000	AU 3369900 A	04-09-2000
			WO 0049035 A1	24-08-2000
WO 9629097	A	26-09-1996	WO 9629097 A1	26-09-1996
WO 0052188	A	08-09-2000	AU 2926000 A	21-09-2000
			WO 0052188 A1	08-09-2000
WO 0011154	A	02-03-2000	AU 5544499 A	14-03-2000
			CA 2340335 A1	02-03-2000
			EP 1105470 A1	13-06-2001
			JP 2002532058 T	02-10-2002
			WO 0011154 A1	02-03-2000
			US 2002076808 A1	20-06-2002
WO 03012082	A	13-02-2003	WO 03012082 A2	13-02-2003