

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
5 August 2004 (05.08.2004)

PCT

(10) International Publication Number  
**WO 2004/066183 A3**

- (51) International Patent Classification<sup>7</sup>: **G06F 19/00**, C12Q 1/68
- (21) International Application Number:  
PCT/IB2004/000620
- (22) International Filing Date: 22 January 2004 (22.01.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
0301459.4 22 January 2003 (22.01.2003) GB  
0317991.8 31 July 2003 (31.07.2003) GB
- (71) Applicant (for all designated States except US): **EUROPEAN MOLECULAR BIOLOGY LABORATORY** [DE/DE]; Postfach 102209 (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **COHEN, Stephen** [CA/DE]; European Molecular Biology Laboratory, Postfach 102209, 69012 Heidelberg (DE). **BRENNECKE, Julius** [DE/DE]; European Molecular Biology Laboratory, Postfach 102209, 69012 Heidelberg (DE). **RUSSELL, Robert, B.** [CA/DE]; European Molecular Biology Laboratory, Postfach 102209, 69012 Heidelberg (DE). **STARK, Alexander** [DE/DE]; European Molecular Biology Laboratory, Postfach 102209, 69012 Heidelberg (DE).
- (74) Agent: **GOODFELLOW, Hugh, Robin**; Carpmaels & Ransford, 43-45 Bloomsbury Square, London WC1A 2RA (GB).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, 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, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (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 (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, 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 receipt of amendments
- (88) Date of publication of the international search report:  
2 December 2004
- 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.



WO 2004/066183 A3

(54) Title: MICRORNA

(57) Abstract: The invention relates to computational methods of identifying novel microRNA (miRNA) molecules and novel targets for miRNA molecules and the microRNA molecules and targets identified by such methods.

## INTERNATIONAL SEARCH REPORT

In International Application No  
PCT/IB2004/000620

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 G06F19/00 C12Q1/68

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 7 G06F 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)

SEQUENCE SEARCH, EPO-Internal, WPI Data, EMBASE, BIOSIS, INSPEC, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEE R C ET AL: "An extensive class of small RNAs in <i>Caenorhabditis elegans</i> ." SCIENCE. UNITED STATES 26 OCT 2001, vol. 294, no. 5543, 26 October 2001 (2001-10-26), pages 862-864, XP002284273 ISSN: 0036-8075 abstract page 862, column 3, paragraph 2 -page 863, column 1, paragraph 2 figure 2; table 1 --- -/--	1-15, 24-27

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

7 September 2004

Date of mailing of the international search report

05. 10. 2004

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

Favre, N

## INTERNATIONAL SEARCH REPORT

 International Application No  
 PCT/IB2004/000620

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LAGOS-QUINTANA MARIANA ET AL: "Identification of tissue-specific microRNAs from mouse." CURRENT BIOLOGY: CB. ENGLAND 30 APR 2002, vol. 12, no. 9, 30 April 2002 (2002-04-30), pages 735-739, XP002284274 ISSN: 0960-9822 page 737, column 1, paragraph 2 -column 2, paragraph 2; figure 2; table 1 ---	1-15, 24-27
X	HIPFNER DAVID R ET AL: "The bantam gene regulates Drosophila growth." GENETICS. UNITED STATES AUG 2002, vol. 161, no. 4, August 2002 (2002-08), pages 1527-1537, XP002284380 ISSN: 0016-6731 cited in the application abstract; figure 1 ---	24-27
A	MOSS ERIC G ET AL: "MicroRNAs: something new under the sun." CURRENT BIOLOGY: CB. ENGLAND 15 OCT 2002, vol. 12, no. 20, 15 October 2002 (2002-10-15), pages R688-R690, XP002284275 ISSN: 0960-9822 the whole document ---	1-15, 24-27
A	LAU N C ET AL: "An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans." SCIENCE. UNITED STATES 26 OCT 2001, vol. 294, no. 5543, 26 October 2001 (2001-10-26), pages 858-862, XP002284276 ISSN: 0036-8075 the whole document ---	1-15, 24-27
A	SERRA M J ET AL: "Predicting thermodynamic properties of RNA." METHODS IN ENZYMOLOGY. UNITED STATES 1995, vol. 259, 1995, pages 242-261, XP009032022 ISSN: 0076-6879 cited in the application the whole document ---	1-15, 24-27
	-/--	

## INTERNATIONAL SEARCH REPORT

 International Application No  
 PCT/IB2004/000620

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EDDY S R: "Profile hidden Markov models" METHODS IN ENZYMOLOGY, 'Online! 1998, XP002194730 Retrieved from the Internet: <URL:ftp://ftp.genetics.wustl.edu/pub/eddy /papers/hmmreview-bioinformatics -98.pdf> 'retrieved on 2002-03-28! cited in the application the whole document	1-15, 24-27
P,X	BRENECKE J ET AL: "Towards a complete description of the microRNA complement of animal genomes" GENOME BIOLOGY 2003 UNITED KINGDOM, vol. 4, no. 9, 2003, pages 228-228.3, XP009032047 ISSN: 1465-6906 page 228.2, column 1, paragraph 4 -column 2, paragraph 3	1-15, 24-27
P,X	AMBROS VICTOR ET AL: "A uniform system for microRNA annotation." RNA (NEW YORK), vol. 9, no. 3, March 2003 (2003-03), pages 277-279, XP009032091 ISSN: 1355-8382 the whole document	1-15, 24-27
P,X	GRAD Y ET AL: "Computational and experimental identification of C. elegans microRNAs" MOLECULAR CELL 01 MAY 2003 UNITED STATES, vol. 11, no. 5, 1 May 2003 (2003-05-01), pages 1253-1263, XP009032050 ISSN: 1097-2765 "Experimental Procedures" page 1253, column 2, paragraph 4 -page 1254, column 1, paragraph 1	1-15, 24-27
P,X	LIM LEE P ET AL: "Vertebrate microRNA genes." SCIENCE (WASHINGTON D C), vol. 299, no. 5612, 7 March 2003 (2003-03-07), page 1540 XP002284278 ISSN: 0036-8075 (ISSN print) the whole document	1-15, 24-27

-/--

## INTERNATIONAL SEARCH REPORT

 Int. Application No  
 PCT/IB2004/000620

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	BRENNECKE JULIUS ET AL: "bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila." CELL. UNITED STATES 4 APR 2003, vol. 113, no. 1, 4 April 2003 (2003-04-04), pages 25-36, XP001181836 ISSN: 0092-8674 figure 1	24-27
P,A	the whole document	1-15
P,A	NELSON P ET AL: "The microRNA world: small is mighty" TIBS TRENDS IN BIOCHEMICAL SCIENCES, ELSEVIER PUBLICATION, CAMBRIDGE, EN, vol. 28, no. 10, October 2003 (2003-10), pages 534-540, XP004464445 ISSN: 0968-0004 the whole document	1-15, 24-27
P,A	WO 03/065281 A (HEALTH RESEARCH INC ;DING YE (US); LAWRENCE CHARLES E (US)) 7 August 2003 (2003-08-07) the whole document	1-15, 24-27
P,X	claims 12-16	16-29
E	WO 2004/009779 A (BOWMAN LEWIS HOWARD ;UNIV SOUTH CAROLINA (US); MALLORY ALLISON (US)) 29 January 2004 (2004-01-29) the whole document	1-15, 24-27
A	RHOADES MATTHEW W ET AL: "Prediction of plant microRNA targets." CELL. 23 AUG 2002, vol. 110, no. 4, 23 August 2002 (2002-08-23), pages 513-520, XP002295225 ISSN: 0092-8674 cited in the application the whole document	16-29
P,X	LEWIS BENJAMIN P ET AL: "Prediction of mammalian microRNA targets." CELL. 26 DEC 2003, vol. 115, no. 7, 26 December 2003 (2003-12-26), pages 787-798, XP002295226 ISSN: 0092-8674 figure 1	16-29
	-/--	

## INTERNATIONAL SEARCH REPORT

In International Application No  
PCT/IB2004/000620

## 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	STARK A ET AL: "IDENTIFICATION OF DROSOPHILA MICRORNA TARGETS" PLOS BIOLOGY, XX, XX, vol. 1, no. 3, December 2003 (2003-12), page 397 XP009032040 ISSN: 1544-9173 figure 2	16-29
P,X	----- ENRIGHT ANTON J ET AL: "MicroRNA targets in Drosophila." GENOME BIOLOGY 2003, vol. 5, no. 1, 2003, pages 1-26, XP002295230 ISSN: 1465-6914 the whole document	16-29
T	----- KIRIAKIDOU M ET AL: "A COMBINED COMPUTATIONAL-EXPERIMENTAL APPROACH PREDICTS HUMAN MICRORNA TARGETS" GENES AND DEVELOPMENT, COLD SPRING HARBOR LABORATORY PRESS, NEW YORK, US, vol. 18, no. 10, 15 May 2004 (2004-05-15), pages 1165-1178, XP009032049 ISSN: 0890-9369 the whole document -----	16-29

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IB2004/000620

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 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 III Observations where unity of invention is lacking (Continuation of item 3 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.:  
  
1-23 (completely) and 24-29 (partially)
  
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: 1-15 (completely) and claims 24-27 (partially)

Invention 1

This invention relates to a (computer-implemented) method for identifying microRNA (miRNA), to isolated miRNA molecules identified or identifiable by said method, and to an isolated miRNA molecule that comprises or consists of SEQ ID NO:1.

2. Claims: 24-27 (partially)

Inventions 2-241

These inventions relate respectively to the 240 different isolated miRNA molecules listed in Table 1.

3. Claims: 16-23 (completely) and 24, 28 and 29 (all partially)

Invention 242

This invention relates to a (computer-implemented) method for identifying the target molecule of a miRNA of interest, to the isolated target molecules identified or identifiable by said method, and to an isolated target molecule of a miRNA of interest that comprises or consists of SEQ ID NO:2.

4. Claims: 24, 28 and 29 (all partially)

Invention 243

This invention relates to an isolated target molecule of a miRNA of interest that comprises or consists of SEQ ID NO:3.

5. Claims: 30-33



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention 244

This invention relates to an assay to measure and visualise miRNA expression, and to transgenic animals or plants useful in the assay of claim 30.

6. Claims: 34-38

Invention 245

This invention relates to a method of creating a conserved 3' UTR database for a candidate organism.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/IB2004/000620

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 03065281	A	07-08-2003	US	2004002083 A1	01-01-2004
			WO	03065281 A1	07-08-2003
WO 2004009779	A	29-01-2004	WO	2004009779 A2	29-01-2004