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Declarations under Rule 4.17:

— as to the identity of the inventor (Rule 4.17(i))

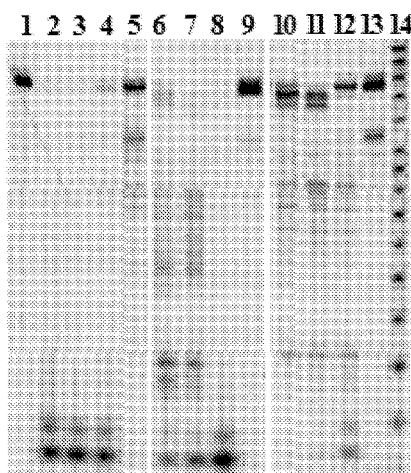
Published:

— with international search report (Art. 21(3))

[Continued on next page]

(54) Title: SEQUENCE-SPECIFIC ENGINEERED RIBONUCLEASE H AND THE METHOD FOR DETERMINING THE SEQUENCE PREFERENCE OF DNA-RNA HYBRID BINDING PROTEINS

Fig. 3



(57) Abstract: The subject of the invention is the ribonuclease which cleaves RNA strand in DNA-RNA hybrids, wherein ribonuclease comprises fusion protein comprising catalytic domain of RNase HI (RNase HI) or derivative thereof with a zinc finger DNA-RNA hybrid binding domain, and wherein the zinc finger binding domain has the ability to bind to specific sequences in the DNA-RNA hybrid. The invention also relates to new method for determination of the sequence preference of DNA-RNA hybrid binding protein(s) or its domain(s) and allows determining the sequence recognized by sequence specific binding protein in the DNA-RNA hybrid.



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- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))* (88) **Date of publication of the international search report:**
7 March 2013

INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2012/050019

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/22
ADD.

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)
C12N

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 practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DURAI S ET AL: "Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells", NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 33, no. 18, 26 October 2005 (2005-10-26), pages 5978-5990, XP002403200, ISSN: 0305-1048 the whole document ----- -/--	1-5,9-15



Further documents are listed in the continuation of Box C.



See patent family 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 application or patent 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

14 December 2012

Date of mailing of the international search report

03/01/2013

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/PL2012/050019

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Y SHI ET AL: "Specific DNA-RNA hybrid binding by zinc finger proteins", SCIENCE, vol. 268, no. 5208, 14 April 1995 (1995-04-14), pages 282-284, XP55039658, ISSN: 0036-8075, DOI: 10.1126/science.7536342 cited in the application the whole document	1-5,9-15
A	MARCIN NOWOTNY ET AL: "Crystal Structures of RNase H Bound to an RNA/DNA Hybrid: Substrate Specificity and Metal-Dependent Catalysis", CELL, vol. 121, no. 7, 1 July 2005 (2005-07-01), pages 1005-1016, XP55039777, ISSN: 0092-8674, DOI: 10.1016/j.cell.2005.04.024 the whole document	1-15
Y	WO 2007/014181 A2 (UNIV JOHNS HOPKINS [US]; CHANDRASEGARAN SRINIVASAN [US]) 1 February 2007 (2007-02-01) cited in the application the whole document	1-5,9-15
Y	WO 2010/076939 A1 (TOOLGEN INC [KR]; KIM JIN SOO [KR]; KIM HYE JOO [KR]) 8 July 2010 (2010-07-08) cited in the application the whole document	1-5,9-15
X	WO 2011/022427 A1 (ARCHEMIX CORP [US]; SCHAUB ROBERT G [US]; MCGINNESS KATHLEEN [US]; NEL) 24 February 2011 (2011-02-24) the whole document	16-23
A	WO 2009/126632 A1 (ARCHEMIX CORP [US]; THOMPSON KRISTIN [US]; WILSON CHARLES [US]; ZHU SH) 15 October 2009 (2009-10-15) the whole document	16-23
A	US 5 270 163 A (GOLD LARRY [US] ET AL) 14 December 1993 (1993-12-14) the whole document	16-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/PL2012/050019

Box No. 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 No. 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 fees, this Authority did not invite payment of additional fees.
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
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 and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ 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

relates to an ribonuclease as defined in claim 1, which cleaves DNA-RNA hybrids;

2. claims: 16-23

relates to a method of determining the sequence preference of DNA-RNA hybrid binding protein as defined in claim 16;

3. claims: 24-26

relates to a method of obtaining a library of DNA-RNA hybrids as defined in claim 24.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/PL2012/050019

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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US 5270163 A	14-12-1993	NONE	