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AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,  
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DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,  
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,  
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,  
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**Declarations under Rule 4.17:**

— as to applicant's entitlement to apply for and be granted a  
patent (Rule 4.17(ii))

**Published:**

— with international search report (Art. 21(3))  
— before the expiration of the time limit for amending the  
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(88) Date of publication of the international search report:  
10 November 2016

(54) Title: METHODS OF USING OLIGONUCLEOTIDE-GUIDED ARGONAUTE PROTEINS

(57) Abstract: The invention relates to the use of Argonaute polypeptide:guide molecule complexes as fast and specific nucleic acid probes, as specific, nucleic acid-guided restriction enzymes for DNA and RNA substrates, and as a means to detect RNA-protein interactions, RNA detection, DNA detection, and RNA depletion. Using such Argonaute polypeptide:guide molecule complexes enables fast and specific detection, purification, and enzymatic activity.



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/025724

### Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a. ☐ forming part of the international application as filed:
    - ☐ in the form of an Annex C/ST.25 text file.
    - ☐ on paper or in the form of an image file.
  - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
    - ☒ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
    - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2016/025724

### 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.:
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.:

1-52, 57-81

#### 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.

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2016/025724

A. CLASSIFICATION OF SUBJECT MATTER  
INV. C12N15/10  
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)

EP0-Internal, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                 | Relevant to claim No.  |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| X         | <p>WO 2014/189628 A1 (CARIBOU BIOSCIENCES INC [US]) 27 November 2014 (2014-11-27)<br/> paragraph [0005] - paragraph [0006]<br/> paragraph [0016]<br/> paragraph [0252] - paragraph [0273]<br/> example 40<br/> figures 34-36</p> <p style="text-align: center;">-----<br/>-/--</p> | <p>1-52,<br/>57-81</p> |



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

26 July 2016

Date of mailing of the international search report

11/10/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
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Tel. (+31-70) 340-2040,  
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Authorized officer

Ulbrecht, Matthias

# INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/025724

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                         | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | DAAN C. SWARTS ET AL: "DNA-guided DNA interference by a prokaryotic Argonaute", NATURE, vol. 507, no. 7491, 16 February 2014 (2014-02-16), pages 258-261, XP055156328, ISSN: 0028-0836, DOI: 10.1038/nature12971 page 259, right-hand column, last paragraph - page 260, left-hand column, paragraph 2<br>Methods: Activity assays<br>figures 3,4<br>----- | 1-52,<br>57-81        |
| A         | G. SHENG ET AL: "Structure-based cleavage mechanism of Thermus thermophilus Argonaute DNA guide strand-mediated DNA target cleavage", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 111, no. 2, 27 December 2013 (2013-12-27), pages 652-657, XP055156327, ISSN: 0027-8424, DOI: 10.1073/pnas.1321032111<br>the whole document<br>-----            | 1-52,<br>57-81        |
| A         | DAAN C SWARTS ET AL: "The evolutionary journey of Argonaute proteins", NATURE STRUCTURAL AND MOLECULAR BIOLOGY, vol. 21, no. 9, 5 September 2014 (2014-09-05), pages 743-753, XP055287457, US<br>ISSN: 1545-9993, DOI: 10.1038/nsmb.2879<br>the whole document<br>-----                                                                                    | 1-52,<br>57-81        |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/025724

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| W0 2014189628 A1                          | 27-11-2014          | EP 2984175 A1              | 17-02-2016          |
|                                           |                     | US 2015089681 A1           | 26-03-2015          |
|                                           |                     | W0 2014189628 A1           | 27-11-2014          |
| -----                                     |                     |                            |                     |

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-52, 57-81

A method of cleaving an RNA or DNA molecule as defined in claim 1 and a kit as defined in claim 57.

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2. claims: 53-56

A method of subcloning a desired double stranded nucleic acid fragment as defined in claim 53.

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3. claims: 82-124

A method of recruiting an Argonaute polypeptide to a heterologous target RNA or DNA sequence as defined in claim 82.

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4. claims: 125-146

A method of identifying an RNA binding polypeptide as defined in claim 125.

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