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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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(54) Title: MODIFYING THE SPECIFICITY OF NON-CODING RNA MOLECULES FOR SILENCING GENES IN EUKARYOTIC CELLS

(57) Abstract: A method of modifying a gene encoding or processed into a non-coding RNA molecule having no RNA silencing activity in a eukaryotic cell, wherein the gene encoding or processed into the non-coding RNA molecule is positioned in a coding gene, is disclosed. The method comprising introducing into the eukaryotic cell a DNA editing agent conferring a silencing specificity of the non-coding RNA molecule towards a target RNA of interest. Target RNA of interest include, for example, a transcript of a gene selected from the group consisting of a housekeeping gene, a dominant gene, a gene comprising a high copy number, and a gene associated with cell apoptosis. Methods comprising DNA or RNA editing agents which elicit base editing are also disclosed.



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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/11 C12N15/113
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, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2005/203047 A1 (THOMANN ULRICH [US] ET AL) 15 September 2005 (2005-09-15) claims 1-4, 19, 20; figures 3-6 -----	1,9-50
Y	Jianping Zhou ET AL: "CRISPR-Cas9 Based Genome Editing Reveals New Insights into MicroRNA Function and Regulation in Rice", Frontiers in plant science, vol. 8 13 September 2017 (2017-09-13), page 1598, XP55528166, Switzerland DOI: 10.3389/fpls.2017.01598 Retrieved from the Internet: URL:file:///C:/Users/AB03938/AppData/Local/Temp/fpls-08-01598-2.pdf page 8; figure 4 ----- -/--	1,4,7-50



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

3 September 2020

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11/09/2020

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/052241

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	T. GUTSCHNER ET AL: "Noncoding RNA gene silencing through genomic integration of RNA destabilizing elements using zinc finger nucleases", GENOME RESEARCH, vol. 21, no. 11, 15 August 2011 (2011-08-15), pages 1944-1954, XP55197552, ISSN: 1088-9051, DOI: 10.1101/gr.122358.111 figures 2,3	1,9-50
Y	----- ELENA SENÍS ET AL: "TALEN/CRISPR-mediated engineering of a promoterless anti-viral RNAi hairpin into an endogenous miRNA locus", NUCLEIC ACIDS RESEARCH, vol. 45, no. 1, 9 September 2016 (2016-09-09), page e3, XP55541534, ISSN: 0305-1048, DOI: 10.1093/nar/gkw805 figures 2,3 -& Elena Senís ET AL: "SUPPLEMENTARY INFORMATION for TALEN/CRISPR-mediated engineering of a promoterless anti-viral RNAi hairpin into an endogenous miRNA locus", 9 September 2016 (2016-09-09), XP55696419, Retrieved from the Internet: URL:https://academic.oup.com/nar/article/45/1/e3/2871108#supplementary-data [retrieved on 2020-05-18] the whole document	1,9-50
Y	----- WO 2018/183878 A1 (PIONEER HI BRED INT [US]) 4 October 2018 (2018-10-04)	4,7-50
A	claims 1,2,19,20	1
A	----- JOLLY BASAK ET AL: "Targeting Non-Coding RNAs in Plants with the CRISPR-Cas Technology is a Challenge yet Worth Accepting", FRONTIERS IN PLANT SCIENCE, vol. 6, 19 November 2015 (2015-11-19), page 1001, XP55528168, DOI: 10.3389/fpls.2015.01001 figures 1,2	1,9-50
Y	----- WO 2019/005884 A1 (BROAD INST INC [US]; MASSACHUSETTS INST TECHNOLOGY [US] ET AL.) 3 January 2019 (2019-01-03) paragraph [0336]; claims 1-8 page 534	4,7-50
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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JIE YANG ET AL: "CRISPR/Cas9-mediated noncoding RNA editing in human cancers", RNA BIOLOGY, vol. 15, no. 1, 9 November 2017 (2017-11-09), pages 35-43, XP55697923, ISSN: 1547-6286, DOI: 10.1080/15476286.2017.1391443 page 36, right-hand column - page 37, right-hand column; figure 2; table 1 -----	4,7-50
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A	REES HOLLY A ET AL: "Base editing: precision chemistry on the genome and transcriptome of living cells", NATURE REVIEWS GENETICS, NATURE PUBLISHING GROUP, GB, vol. 19, no. 12, 15 October 2018 (2018-10-15), pages 770-788, XP036637435, ISSN: 1471-0056, DOI: 10.1038/S41576-018-0059-1 [retrieved on 2018-10-15] the whole document -----	4,7-50
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International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	YUMENG WANG ET AL: "When MicroRNAs Meet RNA Editing in Cancer: A Nucleotide Change Can Make a Difference", BIOESSAYS, vol. 40, no. 2, 27 December 2017 (2017-12-27), page 1700188, XP55720780, GB ISSN: 0265-9247, DOI: 10.1002/bies.201700188 table 1 -----	4,7-50
A	Kunling Chen ET AL: "Annual Review of Plant Biology CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture 2.3. CRISPR/Cas9-Mediated Manipulations Beyond Double-Strand Breaks Controlling Agricultural Invasive Species with CRISPR/Cas-Based Gene Drives", Annu. Rev. Plant Biol, 5 March 2019 (2019-03-05), pages 46667-97, XP55697931, DOI: 10.1146/annurev-arplant-050718-Retrieved from the Internet: URL:https://www.annualreviews.org/doi/pdf/10.1146/annurev-arplant-050718-100049 page 682; figure 5 -----	4,7-50
X,P	WO 2019/058253 A1 (TROPIC BIOSCIENCES UK LTD [GB]) 28 March 2019 (2019-03-28) claims 1,2,43-49; examples 1-6,8,9,12 -----	1,9-50
X,P	WO 2019/058255 A1 (TROPIC BIOSCIENCES UK LTD [GB]) 28 March 2019 (2019-03-28) claims 1,2,39-50; examples 1,8,11 -----	1,9-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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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, 4(completely); 7-50(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 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(completely); 9-50(partially)

A method of modifying a gene encoding or processed into a non-coding RNA molecule having no RNA silencing activity in a eukaryotic cell, wherein the gene encoding or processed into the non-coding RNA molecule is positioned in a coding gene, the method comprising introducing into the eukaryotic cell a DNA editing agent conferring a silencing specificity of said non-coding RNA molecule towards a target RNA of interest, thereby modifying the gene encoding or processed into the non-coding RNA molecule.

2. claims: 2(completely); 9-50(partially)

A method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a eukaryotic cell, wherein the gene encoding or processed into the non-coding RNA molecule is positioned in a coding gene, the method comprising introducing into the eukaryotic cell a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding or processed into the RNA silencing molecule.

3. claims: 3(completely); 7-50(partially)

A method of modifying a gene encoding or processed into a non-coding RNA molecule having no RNA silencing activity in a eukaryotic cell, the method comprising introducing into the eukaryotic cell a DNA or RNA editing agent conferring a silencing specificity of said non-coding RNA molecule towards a target RNA of interest, wherein said DNA or RNA editing agent elicits base editing, thereby modifying the gene encoding or processed into the non-coding RNA molecule.

4. claims: 4(completely); 7-50(partially)

A method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a eukaryotic cell, the method comprising introducing into the eukaryotic cell a DNA or RNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, and wherein said DNA or RNA editing agent elicits base editing, thereby modifying the gene encoding or processed into the RNA silencing molecule.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. claims: 5(completely); 7-50(partially)

A method of modifying a gene encoding or processed into a non-coding RNA molecule having no RNA silencing activity in a eukaryotic cell, the method comprising introducing into the eukaryotic cell a DNA editing agent conferring a silencing specificity of said non-coding RNA molecule towards a target RNA of interest, wherein said target RNA of interest is a transcript of a gene selected from the group consisting of a housekeeping gene, a dominant gene, a gene comprising a high copy number, and a gene associated with cell apoptosis, thereby modifying the gene encoding or processed into the non-coding RNA molecule.

6. claims: 6(completely); 7-50(partially)

A method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a eukaryotic cell, the method comprising introducing into the eukaryotic cell a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, wherein said second target RNA is a transcript of a gene selected from the group consisting of a housekeeping gene, a dominant gene, a gene comprising a high copy number, and a gene associated with cell apoptosis, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding or processed into the RNA silencing molecule.
