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- (72) **Inventor; and**
- (75) **Inventor/Applicant (for US only):** MCGONIGLE, Brian [US/US]; 1707 N. Union Street, Wilmington, Delaware 19806-2501 (US).
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(54) **Title:** DOWN-REGULATION OF GENE EXPRESSION USING ARTIFICIAL MICRORNAS

(57) **Abstract:** Isolated nucleic acid fragments comprising precursor miRNA, and artificial miRNAs and their use in down-regulating gene expression are described.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/087136

A. CLASSIFICATION OF SUBJECT MATTER INV. C12N15/82 A01H5/00				
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 AOIH				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Lkjctronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal , WPI Data, BIOSIS, EMBASE, EMBL, Sequence Search				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No		
X	WO 2006/044322 A (UNIV ROCKEFELLER [US]i, TOBODA JOSE REYES [MX]; ZHANG XIUREN [US]; SOYA) 27 April 2006 (2006-04-27) examples 9,11-17 -----	1-6		
A	ZHANG B ET AL: "Identification of 188 conserved maize microRNAs and their targets" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 580, no. 15, 26 June 2006 (2006-06-26), pages 3753-3762, XP025171330 ISSN: 0014-5793 [retrieved on 2006-06-26] cited in the application table 1 ----- -/-	1-6		
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C </td> <td style="width: 50%; border: none;"> <input checked="" type="checkbox"/> See patent family annex </td> </tr> </table>			<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C	<input checked="" type="checkbox"/> See patent family annex
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C	<input checked="" type="checkbox"/> 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 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 <p style="text-align: center;">18 March 2009</p>	Date of mailing of the international search report <p style="text-align: center;">30/07/2009</p>			
Name and mailing address of the ISA/ European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Fax (+31-70) 340-3016	Authorized officer <p style="text-align: center;">Bucka, Alexander</p>			

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/087136

(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	<p>DATABASE EMBL [Online] 4 August 2005 (2005-08-04), "ZM_BFb0083D07.r ZM_BFb Zea mays cDNA 5', mRNA sequence." XP002519745 retrieved from EBI accession no. EMBL:DR963981 Database accession no. DR963981 the whole document</p> <p style="text-align: center;">-----</p>	1-6
A	<p>WO 2004/009779 A (UNIV SOUTH CAROLINA [US]; VANCE BOWMAN VICKI [US]; BOWMAN LEWIS HOWARD) 29 January 2004 (2004-01-29) examples 3-5</p> <p style="text-align: center;">-----</p>	1-6
A	<p>NIU QI-WEN ET AL: "Expression of artificial microRNAs in transgenic Arabidopsis thaliana confers virus resistance" NATURE BIOTECHNOLOGY,, vol. 24, no. 11, 1 November 2006 (2006-11-01), pages 1420-1428, XP002514067 the whole document</p> <p style="text-align: center;">-----</p>	1-6
A	<p>WILLMANN ET AL: "Conservation and evolution of miRNA regulatory programs in plant development" CURRENT OPINION IN PLANT BIOLOGY, QUADRANT SUBSCRIPTION SERVICES, GB, vol. 10, no. 5, 4 October 2007 (2007-10-04), pages 503-511, XP022286649 ISSN: 1369-5266 the whole document</p> <p style="text-align: center;">-----</p>	1-6
A	<p>SCHWAB REBECCA ET AL: "Highly specific gene silencing by artificial microRNAs in Arabidopsis" PLANT CELL, vol. 18, no. 5, May 2006 (2006-05), pages 1121-1133, XP002519865 ISSN: 1040-4651 the whole document</p> <p style="text-align: center;">-----</p>	1-6
A	<p>PALATNIK JAVIER F ET AL: "Sequence and expression differences underlie functional specialization of Arabidopsis MicroRNAs miR159 and miR319" DEVELOPMENTAL CELL, vol. 13, no. 1, July 2007 (2007-07), pages 115-125, XP002519866 ISSN: 1534-5807 the whole document</p> <p style="text-align: center;">-----</p>	1-6

INTERNATIONAL SEARCH REPORT

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 allsearchable 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.:

see annex

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 POT/ISA/ 210

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

1. claims: 1-6

An isolated nucleic acid fragment comprising a precursor miRNA said precursor miRNA corresponding substantially to the deoxyribonucleotide sequence set forth in SEQ ID NO:11 (i) wherein nucleotides 430 to 450 of SEQ ID NO:11 are replaced by a first variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides depending upon the target sequence whose expression is to be reduced and (ii) further wherein nucleotides 244 to 264 of SEQ ID NO:11 are replaced by a second variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides, said second variable nucleotide subsequence being capable of hybridizing to the first variable subsequence of the precursor miRNA;
plants comprising said nucleic acid fragment;
methods for reducing expression of a target sequence in a plant cell comprising using said nucleic acid fragment.

2. claims: 7-12

An isolated nucleic acid fragment comprising a precursor miRNA said precursor miRNA corresponding substantially to the deoxyribonucleotide sequence set forth in SEQ ID NO:12 (i) wherein nucleotides 94 to 114 SEQ ID NO:12 are replaced by a first variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides depending upon the target sequence whose expression is to be reduced and (ii) further wherein nucleotides 163 to 183 of SEQ ID NO:12 are replaced by a second variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides, said second variable nucleotide subsequence being capable of hybridizing to the first variable subsequence of the precursor miRNA;
plants comprising said nucleic acid fragment;
methods for reducing expression of a target sequence in a plant cell comprising using said nucleic acid fragment.

3. claims: 13-18

FURTHER INFORMATION CONTINUED FROM PCT/ASA/ 210

An isolated nucleic acid fragment comprising a precursor miRNA said precursor miRNA corresponding substantially to the deoxyribonucleotide sequence set forth in SEQ ID NO: 13 (i) wherein nucleotides 53 to 73 of SEQ ID NO: 13 are replaced by a first variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides depending upon the target sequence whose expression is to be reduced and (ii) further wherein nucleotides 97 to 117 of SEQ ID NO: 13 are replaced by a second variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides, said second variable nucleotide subsequence being capable of hybridizing to the first variable subsequence of the precursor miRNA;
plants comprising said nucleic acid fragment;
methods for reducing expression of a target sequence in a plant cell comprising using said nucleic acid fragment.

4. claims: 19-24

An isolated nucleic acid fragment comprising a precursor miRNA said precursor miRNA corresponding substantially to the deoxyribonucleotide sequence set forth in SEQ ID NO: 14 (i) wherein nucleotides 110 to 130 of SEQ ID NO: 14 are replaced by a first variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides depending upon the target sequence whose expression is to be reduced and (ii) further wherein nucleotides 184 to 203 of SEQ ID NO: 14 are replaced by a second variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides, said second variable nucleotide subsequence being capable of hybridizing to the first variable subsequence of the , precursor miRNA;
plants comprising said nucleic acid fragment;
methods for reducing expression of a target sequence in a plant cell comprising using said nucleic acid fragment.

5. claims: 25-30

FURTHER INFORMATION CONTINUED FROM POT/ISA/ 210

An isolated nucleic acid fragment comprising a precursor miRNA said precursor miRNA corresponding substantially to the deoxyribonucleotide sequence set forth in SEQ ID NO: 15 (i) wherein nucleotides 83 to 103 of SEQ ID NO: 15 are replaced by a first variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides depending upon the target sequence whose expression is to be reduced and (ii) further wherein nucleotides 172 to 192 of SEQ ID NO: 15 are replaced by a second variable nucleotide subsequence ranging in size from about 19 to about 30 nucleotides, said second variable nucleotide subsequence being capable of hybridizing to the first variable subsequence of the precursor miRNA;

plants comprising said nucleic acid fragment;

methods for reducing expression of a target sequence in a plant cell comprising using said nucleic acid fragment.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2008/087136

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006044322 A	27-04-2006	AU 2005295948 A1	27-04-2006
		BR PI0516874 A	23-09-2008
		CA 2583690 A1	27-04-2006
		EP 1809748 A2	25-07-2007
		JP 2008522585 T	03-07-2008
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WO 2004009779 A	29-01-2004	AU 2003254052 A1	09-02-2004
		CA 2492917 A1	29-01-2004
		EP 1551967 A2	13-07-2005
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