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(54) Title: ENABLING THE USE OF LONG DSRNA FOR GENE TARGETING IN MAMMALIAN AND OTHER SELECTED ANIMAL CELLS

(57) Abstract: The present invention provides a method of enabling the use of long dsRNA for gene silencing in mammalian cells through bacteria, preferably non-pathogenic or therapeutic strains of bacteria. DNA that encodes long double-strand RNAs are transformed into bacteria and processed in the bacterial cells into a mixture of smaller RNA duplexes and then released into the cytoplasm of the target cells, resulting in modulation of gene expression in the target cells. The methods overcome the incompatibility between long strong dsRNA and mammalian cells by eliminating, or mitigating, the non-specific innate immune response. The eukaryotic cells can be mammalian cells or avian cells. The gene of interest can be a mammalian, avian, bacterial, eukaryotic, or viral gene.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/68866

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12P 19/34 (2008.04)

USPC - 435/91.1

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(8): C12P 19/34 (2008.04)

USPC: 435/91.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
435/91.1; 514/44; 536/23.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST(USPT,PGPB,EPAB,JPAB); DialogPRO--Chemical Engineering and Biotechnology Abstracts, INSPEC, NTIS (National Technical Information Service), PASCAL, Current Contents Search, MEDLINE

Search Terms: bacterium, dsRNA, prokaryotic promoter, mammalian gene, dicer, t7, HIV, cancer, k-ras

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2005/0233994 A1 (Kaykas, et al.) 20 October 2005 (20.10.2005), para [0007], [0009], [0040], [0051], [0053], [0121].	1, 31-33 ----- 2-30, 70 and 71
X ✓ ----- Y	WO 2006/066048 A2 (Li, et al.) 22 June 2006 (22.06.2006), pg 2, ln 15-19, pg 3, ln 10-12, pg 5, ln 21-22, pg 6, ln 18, pg 7, ln 33-35, pg 8, ln 1, pg 10, ln 4, pg 10, ln 10, pg 12, ln 13.	34 ----- 7, 25-30, 35, 70 and 71
Y ✓	QIAN, et al., Expression and purification of the carboxyl terminus domain of Schizosaccharomyces pombe dicer in Escherichia coli, Protein Pept Lett (2005) vol 12, no 4, pg 311-4, abstract.	2-6
Y ✓	CLEMENS, et al., Use of double-stranded RNA interference in Drosophila cell lines to dissect signal transduction pathways, Proc Natl Acad Sci (US) (2000) vol 97, no 12, pg 6499-6503, pg 6499, col 1, para 2.	8-13
Y	US 2003/0061626 A1 (Plaetinck, et al.) 27 March 2003 (27.03.2003), para [0009].	14-17
Y	US 6,060,241 A (Corthesy-Theulaz) 09 May 2000 (09.05.2000), col 18, ln 63-64, col 19, ln 1.	16
Y	US 2006/0228800 A1 (Lin, et al.) 12 October 2006 (12.10.2006), para [0019].	18, 19
Y	US 2005/0255086 A1 (Davidson, et al.) 17 November 2005 (17.11.2005), para [0003].	20
Y ✓	WO 2006/078880 A2 (Zhou, et al.) 27 July 2006 (27.07.2006), para [0097].	21

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search

18 December 2008 (18.12.2008)

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Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/68866

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.: 49
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:

Group I: Claims 1-35, 70, and 71 are directed to a bacterium comprising a long double-strand RNA (dsRNA), or a DNA molecule encoding the RNA, or a bacterium comprising a mixture of short RNA.

Group II: Claims 36-45 and 72 are directed to a prokaryotic vector that encodes a long, double-strand RNA (dsRNA) under the control of at least one prokaryotic promoter.

Group III: Claims 46-48 and 50-53 are directed to methods of administering dsRNA.

Group IV: Claims 54-60 are directed to a library comprising a plurality of vectors or bacterial cells.

Group V: Claims 61-69 are directed to methods of investigation using the bacterium.

The common technical features of the listed groups are one or more of long dsRNA, a eukaryotic or viral target, expression in a bacterium, and a prokaryotic promoter. However, this is not an improvement over the prior art of US 2005/0239728 A1 to Satishchandran et al. that teaches dsRNA expression constructs for inhibiting expression of a target eukaryotic (human) gene (abstract and further teaches that the dsRNA may be long dsRNA (para [0024]) and teaches expression of the dsRNA in prokaryotes (para [0065]; [0126]) (para [0060])).

Accordingly, unity of invention is lacking under PCT Rule 13.1.

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 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.: 1-35, 70, and 71

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/US 08/68866

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/058948 A2 (O'Hagan, et al.) 15 July 2004 (15.07.2004), pg 7, ln 30-31	22, 23
Y	US 2004/0191905 A1 (Stevenson, et al.) 30 September 2004 (30.09.2004), para [0007].	24
Y	US 20070020652 A1 (Alvarado, et al.) 25 January 2007 (25.01.2007), para [0045].	35