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[Continued on next page]

(54) Title: LIGATION METHOD EMPLOYING EUKARYOTIC tRNA LIGASE

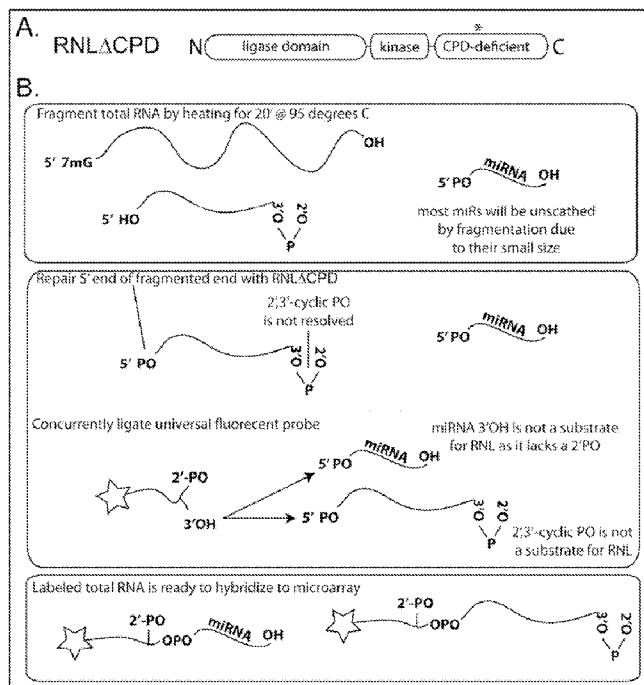


Fig. 2

(57) Abstract: Provided herein is a method of preparing an RNA sample comprising: a) obtaining an RNA sample comprising: i. long RNA molecules that may be unfragmented or fragmented to contain 5'-OH group and a 2'-3'-cyclic phosphate group; and ii. short RNA molecules that comprise a 5' phosphate group and a 3' OH group; and b) contacting the RNA sample with an adaptor comprising either a 2'-PO group and 3'-OH group or a 2',3'-cyclic phosphate group in the presence of a eukaryotic tRNA ligase, thereby producing a ligated RNA sample in which a) the short RNA molecules are selectively ligated to the adaptor or b) the short RNA molecules and long RNA fragments are selectively ligated to the adaptor.





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**A. CLASSIFICATION OF SUBJECT MATTER***C12N 15/10(2006.01)i, C12Q 1/68(2006.01)i, C12Q 1/25(2006.01)i*

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 15/10; C40B 40/08; C40B 50/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal), Pubmed

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LU, C. et al. 'Construction of small RNA cDNA libraries for deep sequencing' Methods. Vol.43(2), pp. 110-117 (October 2007)	1-22
A	See the abstract; Fig. 1.	23,24
Y	ENGLERT, M. and BEIER, H. 'Plant tRNA ligases are multifunctional enzymes that have diverged in sequence and substrate specificity from RNA ligases of other phylogenetic origins' Nucleic Acids Research. Vol.33(1), pp.388-399 (14 January 2005)	1-20
A	See the abstract; Fig. 1; p. 389, left col. para. 3.	21-24
Y	SAUNDERS, R. D. et al. 'PCR amplification of DNA microdissected from a single polytene chromosome band: a comparison with conventional microcloning' Nucleic Acids Research. Vol.17(22), pp.9027-9037 (25 November 1989)	21,22
A	See p.9033, lines 13-25.	1-20,23,24

 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

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"&amp;" document member of the same patent family

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**INTERNATIONAL SEARCH REPORT**

International application No.

**PCT/US2011/065268**

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	APOSTOL, B. L. et al. 'Deletion analysis of a multifunctional yeast tRNA ligase polypeptide. Identification of essential and dispensable functional domains' Journal of Biological Chemistry. Vol.266(12), pp.7445-7455 (25 April 1991) See the abstract.	1-24
A	HO, T. et al. 'A simplified method for cloning of short interfering RNAs from Brassica juncea infected with Turnip mosaic potyvirus and Turnip crinkle carmovirus' Journal of Virological Methods. Vol.136(1-2), pp.217-223 (3 July 2006) See the abstract.	1-24
A	US 2010-0167954 A1 (EARNSHAW, D. J. et al.) 01 July 2010 See the abstract; para. [0039].	1-24

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010-0167954 A1	01.07.2010	EP 2049682 A2 WO 2008-015396 A2 WO 2008-015396 A3	22.04.2009 07.02.2008 27.03.2008