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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, DK, DM, 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, LT, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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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 claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) **Date of publication of the international search report**:
2 April 2015

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(54) **Title**: LINCRNA-DEFICIENT NON-HUMAN ANIMALS

(57) **Abstract**: Genetically modified non-human animals are provided that exhibit a functional lack of one or more lncRNAs. Methods and compositions for disrupting, deleting, and/or replacing lncRNA-encoding sequences are provided. Genetically modified mice that age prematurely are provided. Also provided are cells, tissues and embryos that are genetically modified to comprise a loss-of-function of one or more lncRNAs.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/50178

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/00, C12N 15/87, A61K 48/00 (2014.01)
CPC - C12N 15/907, A61K 48/00, C12N 15/86
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): C12N 15/00, C12N 15/87, A61K 48/00 (2014.01)
CPC: C12N 15/907, A61K 48/00, C12N 15/86

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/455, 435/463, 435/320.1, 514/44R, 435/462

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms: large intergenic non-coding RNAs, linc RNAs, lncRNA , long noncoding RNAs, loss-of-function mutation, disruption, knockout, first exon, reporter, operably linked, promoter, endogenous lncRNA promoter, Pint, lordokyphosis, atrophy, beta-galactosidase, GFP, pluripotent cell, targeted

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Anguera et al. Tsx Produces a Long Noncoding RNA and Has General Functions in the Germline, Stem Cells, and Brain. PLoS Genet (1 September 2011) vol 7, no 9, e1002248, pp 1-14, abstract, pg 3, col 1, para 3, col 2 para 3, pg 7, col 2, para 2, Figs. 1, 3	1-6, 25, 33-35, 37, 39 ---------- 7-11, 18, 26-28, 38, 40
Y	US 2011/0104799 A1 (ECONOMIDES et al.) 5 May 2011 (05.05.2011) para [0002], [0039], [0126], [0128], [0157], [0160], [0161], [0168], [0173], [0175]	7-11, 26-28, 38, 40/38
Y	Khalil et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. PNAS (14 July 2009) vol 106, no 28, pp11677-11672, Fig. 3	18
Y	Rinn et al. Functional Demarcation of Active and Silent Chromatin Domains in Human HOX Loci by Noncoding RNAs. Cell (29 June 2007) vol 129, no 7, pp1311-1323, pg 3 col 2 para 3	40
A	Zhang et al. The lncRNA Malat1 Is Dispensable for Mouse Development but Its Transcription Plays a cis-Regulatory Role in the Adult. Cell Reports (26 July 2012) vol 1, no 1, pp 111-123. abstract.	1

☐ Further documents are listed in the continuation of Box C. ☐

* 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	Date of mailing of the international search report
13 January 2015 (13.01.2015)	29 JAN 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

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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.: 12-17, 19-24, 29-32, 36
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 Extra Sheet to continue **********

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.:

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

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Continuation of Box No. III, Observations where unity of invention is lacking:

The inventions listed as Groups I-II do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features
Group II has the special technical features of a targeting vector, comprising an insert nucleic acid flanked by 5' and 3' homology arms that can undergo homologous recombination with an lncRNA locus of interest, and a method for making a non-human animal comprising contacting a pluripotent cell with a targeting construct comprising an insert nucleic acid flanked by 5' and 3' homology arms; wherein the targeting construct undergoes homologous recombination with the lincRNA locus in a genome of the cell to form a modified pluripotent cell; (b) introducing the modified pluripotent cell into a host embryo; and (c) gestating the host embryo in a surrogate mother, wherein the surrogate mother produces progeny comprising a modified lncRNA locus, wherein said genetic modification results in loss-of-function of the at least one lncRNA, that is not required by Group I.

Common technical features:
Groups I (non-human animal) and II (a targeting vector, and use thereof to modify a lncRNA locus in a pluripotent cell for making a non-human animal) are related, and share the common technical feature of non-human animal comprising in its genome at least one modified long non-coding RNA (lncRNA) locus, wherein the at least one modified lncRNA locus comprises a loss-of-function mutation in a nucleic acid sequence that encodes a lncRNA. However, these shared technical features do not represent a contribution over prior art, because these shared technical features are obviated by the article by Zhang et al., entitled 'The lncRNA Malat1 Is Dispensable for Mouse Development but Its Transcription Plays a cis-Regulatory Role in the Adult' Cell Reports (26 July 2012) vol 1, no 1, pp 111-123, (hereinafter Zhang).

Zhang discloses a mouse non-human animal comprising in its genome at least one modified long non-coding RNA (lncRNA) locus, wherein the at least one modified lncRNA locus comprises a loss-of-function mutation in a nucleic acid sequence that encodes a lncRNA (pg 112, col 1, para 3 'Here, we show that Malat1 is one of the most abundant lncRNAs in mouse liver and brain cortex. ... To examine the role of the Malat1 gene locus, we also established a mouse loss-of-function genetic model.'; p 114, col 1, para 2 'we generated Malat1 mutant mice using homologous recombination in ESCs'; Fig. 3A-C show the modified lncRNA locus, Fig 3D shows the loss-of-function mutation that results in the absence of the Malat1 transcript).

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Groups I and II lack unity of invention under PCT Rule 13.