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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, 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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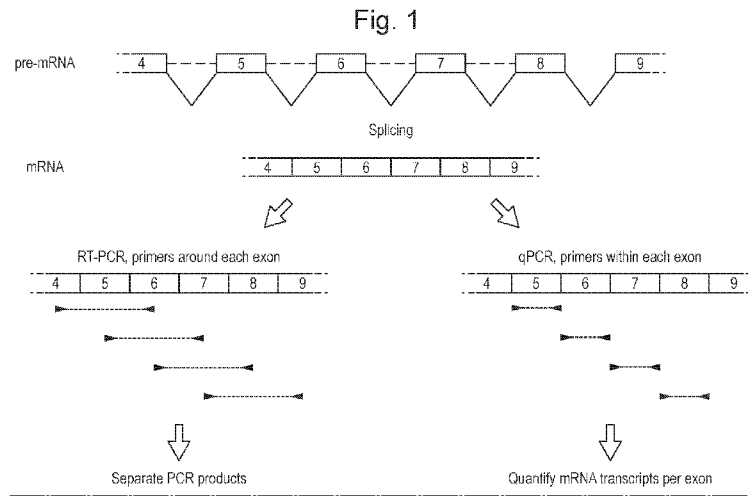
Published:

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- 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))

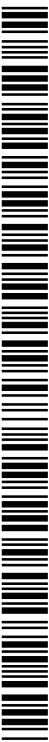
(88) Date of publication of the international search report:

3 March 2016

(54) Title: METHODS FOR CHARACTERIZING ALTERNATIVELY OR ABERRANTLY SPLICED MRNA ISOFORMS



(57) Abstract: The disclosure provides method and kits for characterizing spliced m RNA isoforms. The disclosure also provides methods of screening for mutations and oligonucleotides that modulate splicing.



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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2015/050420

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 A61K48/00 C12N15/11
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)
C12Q A61K 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, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OHNUMA S ET AL: "Cancer-associated splicing variants of the CDCA1 and MSMB genes expressed in cancer cell lines and surgically resected gastric cancer tissues", SURGERY, MOSBY, INC, US, vol. 145, no. 1, 1 January 2009 (2009-01-01), pages 57-68, XP025837299, ISSN: 0039-6060, DOI: 10.1016/J.SURG.2008.08.010 [retrieved on 2008-12-27] abstract; figures 1-3	1,3-11
X	WO 2007/135105 A1 (ACADEMISCH ZIEKENHUIS LEIDEN [NL]; AARTSMA-RUS ANNEMIEKE [NL]; VAN DEU) 29 November 2007 (2007-11-29) paragraphs [0029] - [0031]; figures 1b-1e	1,3-11
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Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"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</p> <p>"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</p> <p>"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</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search 9 September 2015	Date of mailing of the international search report 15/01/2016
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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 Dolce, Luca
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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2015/050420

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>ROB W J COLLIN ET AL: "Mid-frequency DFNA8/12 hearing loss caused by a synonymous TECTA mutation that affects an exonic splice enhancer", EUROPEAN JOURNAL OF HUMAN GENETICS, vol. 16, no. 12, 25 June 2008 (2008-06-25), pages 1430-1436, XP055140967, ISSN: 1018-4813, DOI: 10.1038/ejhg.2008.110 the whole document</p> <p style="text-align: center;">-----</p>	1-11
A	<p>T. YU ET AL: "Using a minigene approach to characterize a novel splice site mutation in human F7 gene causing inherited factor VII deficiency in a Chinese pedigree", HAEMOPHILIA, vol. 15, no. 6, 1 November 2009 (2009-11-01), pages 1262-1266, XP055140970, ISSN: 1351-8216, DOI: 10.1111/j.1365-2516.2009.02064.x the whole document</p> <p style="text-align: center;">-----</p>	1-11
A	<p>YEN M Y ET AL: "Novel Mutations of the OPA1 Gene in Chinese Dominant Optic Atrophy", OPHTHALMOLOGY, J. B. LIPPINCOTT CO., PHILADELPHIA, PA, US, vol. 117, no. 2, 1 February 2010 (2010-02-01), pages 392-396.e1, XP027044165, ISSN: 0161-6420 [retrieved on 2009-12-06] the whole document</p> <p style="text-align: center;">-----</p>	1-11
A	<p>WO 2013/043878 A2 (UNIV GEORGE WASHINGTON [US]) 28 March 2013 (2013-03-28) the whole document</p> <p style="text-align: center;">-----</p>	1-11
A	<p>WO 01/77380 A2 (MEDICAL RES COUNCIL [GB]; WRIGHT ALAN [GB]) 18 October 2001 (2001-10-18) the whole document</p> <p style="text-align: center;">-----</p>	1-11
X	<p>VEISTINEN E ET AL: "Quantification of human Aiolos splice variants by real-time PCR", JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 271, no. 1-2, 20 December 2002 (2002-12-20), pages 113-123, XP004393931, ISSN: 0022-1759, DOI: 10.1016/S0022-1759(02)00370-8 figure 1</p> <p style="text-align: center;">-----</p>	2-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2015/050420

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.:

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.:

1-11

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: 1-11

Method for characterizing a splice form of an mRNA based on performing (i) a flanking exon RT-PCR and (ii) a concomitant exon internal qPCR; method for quantifying an aberrantly spliced isoform of an mRNA; a kit to perform the methods

2. claims: 12-23

methods for identifying a mutation in pre-mRNA that modulates its splicing based on the use of minigenes, methods for screening for compounds that modulate the splicing of a pre-mRNA using minigenes, libraries of minigenes, methods of making antisense oligonucleotides (AON) for modulating the splicing of a mutated pre-mRNA as identified by the method of claim 10

3. claims: 24-31

methods for screening a library of AONs for modulators of the splicing of a pre-mRNA, libraries of AONs

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2015/050420

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007135105	A1	29-11-2007	
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