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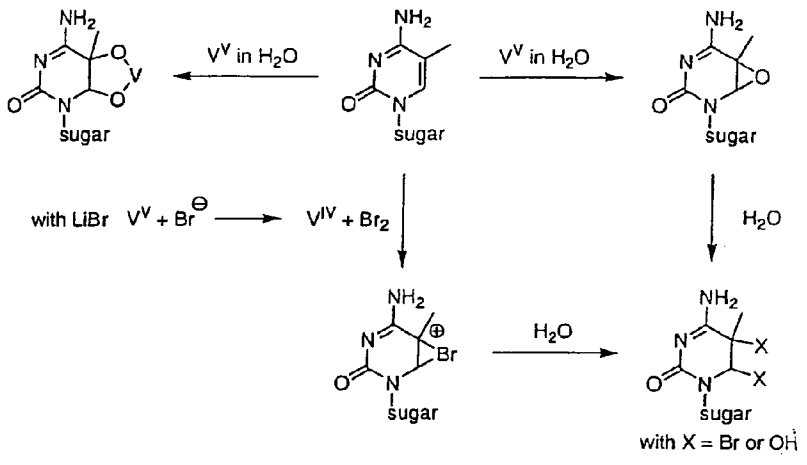
18 June 2009

(54) Title: METHOD FOR DETERMINING METHYLATION AT CYTOSINE RESIDUES

Figure 4



5'-d(AAGTGTCATGAGTTXGATAGGTAAAG-TATT-Fluorescein)

(57) Abstract: The present invention refers to a method and reagent kits for determining methylation at cytosine (dC) residues in nucleic acids, such as DNA.

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INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68

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

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 practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REIN ET AL: "Identifying 5-methylcytosine and related modifications in DNA genomes" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 26, no. 10, 1 January 1998 (1998-01-01), pages 2255-2264, XP002143106 ISSN: 0305-1048	1,2,5, 8-10, 18-20, 22,23
Y	the whole document page 2261, right-hand column - page 2262, left-hand column; figure 3	3,4,6,7, 11-17,21

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[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
15 April 2009	23/04/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pinta, Violaine

INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OKAMOTO A ET AL: "Sequence-selective osmium oxidation of DNA: efficient distinction between 5-methylcytosine and cytosine" ORGANIC AND BIOMOLECULAR CHEMISTRY, ROYAL SOCIETY OF CHEMISTRY, CAMBRIDGE, GB, vol. 4, 21 March 2006 (2006-03-21), pages 1638-1640, XP003006896 ISSN: 1477-0520	18,20
Y	the whole document figures 1-3	3,4,6,7, 11-17,21
X	FRITZSCHE E ET AL: "THE USE OF PERMANGANATE AS A SEQUENCING REAGENT FOR IDENTIFICATION OF 5-METHYLCYTOSINE RESIDUES IN DNA" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 15, no. 14, 24 July 1987 (1987-07-24), pages 5517-5528, XP001207828 ISSN: 0305-1048 the whole document	18,20
X	WO 2006/117161 A (BASF AG [DE]; LUDWIG MAXIMILIANS UNI MUENCHE [DE]; SCHWOEGLER ANJA [DE]) 9 November 2006 (2006-11-09)	19
Y	the whole document page 26, line 33 - page 27, line 6; figure 26	11-16
Y	US 5 217 863 A (CAMPBELL ROBERT DUNCAN [AU] ET AL) 8 June 1993 (1993-06-08) the whole document column 7 - column 8	3,4
A	WO 2006/034264 A (APPLERA CORP [US]; ZON GERALD [US]) 30 March 2006 (2006-03-30) the whole document paragraph [0049]	
A	RUBIN C M ET AL: "Pyrimidine-specific chemical reactions useful for DNA sequencing." NUCLEIC ACIDS RESEARCH 24 OCT 1980, vol. 8, no. 20, 24 October 1980 (1980-10-24), pages 4613-4619, XP002482571 ISSN: 0305-1048 cited in the application the whole document	

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/008875

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BRENA ET AL: "Quantitative assessment of DNA methylation: potential applications for disease diagnosis, classification, and prognosis in clinical settings" JOURNAL OF MOLECULAR MEDICINE, vol. 84, no. 5, 1 May 2006 (2006-05-01), pages 365-377, XP019320445 ISSN: 1432-1440 the whole document	
P,X	BAREYT SÉBASTIAN ET AL: "Selective detection of 5-methylcytosine sites in DNA." ANGEWANDTE CHEMIE (INTERNATIONAL ED. IN ENGLISH) 2008, vol. 47, no. 1, 2008, pages 181-184, XP002515334 ISSN: 1521-3773 the whole document	1-23
T	SULEWSKA ANETTA ET AL: "Detection of DNA methylation in eucaryotic cells." FOLIA HISTOCHEMICA ET CYTOBIOLOGICA / POLISH ACADEMY OF SCIENCES, POLISH HISTOCHEMICAL AND CYTOCHEMICAL SOCIETY 2007, vol. 45, no. 4, 2007, pages 315-324, XP002515335 ISSN: 1897-5631 the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/008875

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 reportcovers only those claims for which fees were paid, specifically claims Nos.:

1-17 (partially), 18-19 (completely)
20-23 (partially) (inventions 1 and 2)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: claims 1-17 (partially), 18 (completely), 20-23 (partially)

methods for determining methylation at cytosine residues in DNA comprising treating a fraction of the sample with a reagent which selectively reacts with 5-methylcytosine residues to obtained modified residues, wherein said reagent is an electrophilic/oxidizing species generated by oxidizing a precursor with an oxidant, reagent kit comprising a reagent which selectively reacts with 5-methylcytosine residues to obtained modified residues, and use of said kit in a method of claims 1-18.

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2. claims: claims 1-17 (partially), 19 (completely), 20-23 (partially)

method for determining methylation at cytosine residues in DNA comprising treating a fraction of the sample with a reagent which selectively reacts with non-methylated cytosine residues to obtained modified residues, wherein said reagent is chosen from hydroxylamine or an N- or O-substituted hydroxylamine, method for determining 5-methylcytosine residues in DNA according to claims 19, 20, 21, reagent kit according to claim 22 wherein the second reagent comprises hydroxylamine or an N- or O-substituted hydroxylamine, and use of said kit in a method of claims 1-19.

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3. claims: claims 1-17 (partially), 19 (completely), 20-23 (partially)

as for invention 2, wherein step (c) comprises using a substituted hydrazine, and the second reagent of the kit comprises a substituted hydrazine.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/008875

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 2006034264 A	30-03-2006	AU 2005286831 A1	30-03-2006
		CA 2581140 A1	30-03-2006
		EP 1791981 A2	06-06-2007
		JP 2008515784 T	15-05-2008