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BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
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RW, 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.

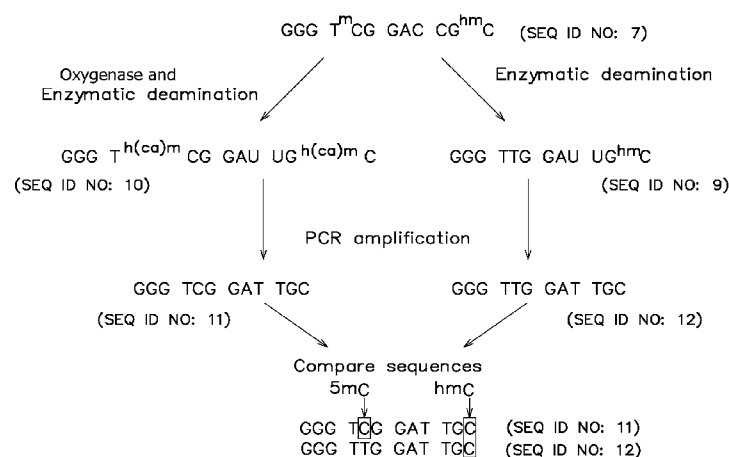
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Published:

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(54) **Title:** METHODS AND COMPOSITIONS FOR DISCRIMINATION BETWEEN CYTOSINE AND MODIFICATIONS THEREOF, AND FOR METHYLOME ANALYSIS

**FIG. 13**

(57) **Abstract:** Compositions and methods are provided for discrimination between cytosine and modifications thereof using cytidine deaminases and/or oxygenases. Variants of wild type cytidine deaminases are described which show reduced bias with respect to adjacent nucleotides upstream of the cytosine. The methods provide a rapid and convenient use of enzymes to obtain methylomes.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/031620

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/78
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)
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, 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.
A	WO 2005/005660 A1 (EPIGENOMICS AG [DE]; GUETIG DAVID [DE]) 20 January 2005 (2005-01-20) p.3, para.2; claims 1-2, 12-15 -----	1-5, 24-29
A	US 2006/210990 A1 (TODD ALISON V [AU] ET AL) 21 September 2006 (2006-09-21) example 1 ----- -/--	1-5, 24-29



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

"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

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/031620

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.:

1-5, 24-29

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/US2013/031620

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	S. M. KINNEY ET AL: "Tissue-specific Distribution and Dynamic Changes of 5-Hydroxymethylcytosine in Mammalian Genomes", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 286, no. 28, 15 July 2011 (2011-07-15), pages 24685-24693, XP055057583, ISSN: 0021-9258, DOI: 10.1074/jbc.M110.217083 abstract; p.24686, col.2, para.2-p.24687, col.1, para.3 -----	1-5, 24-29
X,P	MIAO YU ET AL: "Base-Resolution Analysis of 5-Hydroxymethylcytosine in the Mammalian Genome", CELL, vol. 149, no. 6, 1 June 2012 (2012-06-01), pages 1368-1380, XP055064960, ISSN: 0092-8674, DOI: 10.1016/j.cell.2012.04.027 cited in the application the whole document -----	1-5, 24-29

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/031620

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
WO 2005005660	A1	20-01-2005	AU 2004256178 A1	20-01-2005
			DE 10331107 B3	02-12-2004
			EP 1644521 A1	12-04-2006
			US 2007065824 A1	22-03-2007
			WO 2005005660 A1	20-01-2005

US 2006210990	A1	21-09-2006	CN 1816635 A	09-08-2006
			JP 4891764 B2	07-03-2012
			JP 2007507203 A	29-03-2007
			US 2006210990 A1	21-09-2006

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-5, 24-29

Use of cytidine deaminase and oxygenase for determining the methylation status of a polynucleotide.

2. claims: 6, 7, 14-23, 30-39

Methods for determining the methylation status of a polynucleotide, comprising the use of cytidine deaminase, wherein 5-mC is converted to T and C to U. Mixture comprising cytidine deaminase and DNA polymerase.

3. claims: 8-13

Variant cytidine deaminases as defined by claim 8. Method for determining cytosine preference of cytidine deaminase, as defined in claim 10.
