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(56) Documents Cited:
FLUSBERG BENJAMIN A ET AL:"Direct detection of DNA methylation during single-molecule, real-time sequencing", NATURE METHODS, vol.7, no.6, 1July 2010 (2010-07-01), pages 461-465, XP009142171, NATURE PUBLISHING GROUP, GB ISSN:1548-7105, DOI: 10.1038/NMETH.1459[retrieved on 2010-05-09]
NOMURA AKIKO ET AL:"Discrimination between 5-hydroxymethylcytosine and 5-methylcytosine by a chemically designed peptide.", CHEMICAL COMMUNICATIONS, vol.47, no.29, 21 June 2011 (2011-06-21), pages 8277-8279, XP002689900, CAMBRIDGE,ENGLAND ISSN:1364-548X abstract
GUPTA ROMI ET AL:"Advances in genome-wide DNA methylation analysis.", BIOTECHNIQUES, vol.49, no.4, October 2010 (2010-10), pages iii-xi, XP002689901, ISSN:1940-9818 abstract and p.ix to x
WANUNU MENI ET AL: "Discrimination of methylcytosine from hydroxymethylcytosine in DNA molecules.", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol.133, no.3, 26 January 2011 (2011-01-26), pages 486-492, XP002689902, ISSN:1520-5126 abstract

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(54) Title of the Invention: **Methods for detection of nucleotide modification**
Abstract Title: **Methods for detection of nucleotide modification**

(57) This invention relates to the identification of modified cytosine residues, such as 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC) and 5-formylcytosine (5fC) to be distinguished from cytosine (C) in a sample nucleotide sequence. Methods may comprise oxidising or reducing a first portion of polynucleotides which comprise the sample nucleotide sequence; treating the oxidised or reduced first portion and a second portion of polynucleotides with bisulfite; sequencing the polynucleotides in the first and second portions of the population following steps ii) and iii) to produce first and second nucleotide sequences, respectively and; identifying the residue in the first and second nucleotide sequences which corresponds to a cytosine residue in the sample nucleotide sequence. These methods may be useful, for example in the analysis of genomic DNA and/or of RNA.

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