(54) Title: METHODS AND COMPOSITIONS FOR LONG FRAGMENT READ SEQUENCING

\[ \text{U and 5-methyl C incorporation by MDA} \]
\[ \text{mC} \quad \text{mC} \quad \text{U} \quad \text{U} \]

\[ \text{U and 5 methyl C excision by UDG and McrBC etc.} \]

\[ \text{Nick Translation by Pol I} \]

FIG. 2

(57) Abstract: The present invention is directed to methods and compositions for long fragment read sequencing. The present invention encompasses methods and compositions for preparing long fragments of genomic DNA, for processing genomic DNA for long fragment read sequencing methods, as well as software and algorithms for processing and analyzing sequence data.
— 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))
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C12N15/10 C12Q1/68

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 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched:

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>Y</td>
<td>REICH STEFANIE ET AL: &quot;Combinatorial Domain Hunting: An effective approach for the identification of soluble protein domains adaptable to high-throughput applications&quot;, PROTEIN SCIENCE, vol. 15, no. 10, October 2006 (2006-10), pages 2356-2365, XP00259613, ISSN: 0961-8368 page 2357, right-hand column, paragraph 2 - page 2358, left-hand column, paragraph 1; figure 1</td>
<td>8-11,13, 14</td>
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<td>Y</td>
<td>WO 03/040391 A2 (UNIV LONDON [GB]; BIRKBECK COLLEGE [GB]; CANCER RES INST [GB]; MCALIST) 15 May 2003 (2003-05-15) page 13, line 31 - page 15, line 23; figure 1</td>
<td>8-11,13, 14</td>
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</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

**Date of the actual completion of the international search**

20 May 2011

**Date of mailing of the international search report**

27/05/2011

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) 340-3616

Authorized officer:

Leber, Thomas
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 8.4(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. X 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 fee, 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.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14

   a method of fragmenting a double-stranded target nucleic acid, said method comprising: (a) providing genomic DNA; (b) dividing said DNA into a first tier of separate aliquots; (c) amplifying said DNA in said separate aliquots to form a plurality of amplicons, wherein said amplifying is conducted with a population of dNTPs, wherein said population of dNTPs comprises: (i) a predetermined ratio of dUTP to dTTP, such that a number of thymines in said DNA are replaced by uracils; (ii) a predetermined ratio of 5-methyl dCTP to dCTP, such that a number of cytosines are replaced by 5-methyl cytosines; (d) removing said uracils and said 5-methyl cytosines from said amplicons to form gapped DNA; (e) treating said gapped DNA to translate said gaps until gaps on opposite strands converge, thereby creating blunt-ended DNA fragments, wherein said blunt-ended fragments have less GC bias and less coverage bias as compared to fragments generated in the absence of 5-methyl cytosine, and a method of fragmenting a double-stranded target nucleic acid, said method comprising: (a) providing genomic DNA; (b) dividing said DNA into separate aliquots; (c) amplifying said DNA in said separate aliquots to form a plurality of amplicons, wherein: (i) said amplifying is conducted with a population of dNTPs that comprises dNTP analogs, such that a number of nucleotides in said DNA are replaced by said dNTP analogs; and (ii) said amplifying is conducted in the presence of an additive selected from glyccogen, DMSO, ET SSB, betaine, and any combination thereof; (c) removing said dNTP analogs from said amplicons to form gapped DNA; (d) treating said gapped DNA acid to translate said gaps until gaps on opposite strands converge, thereby creating blunt-ended DNA fragments, wherein said blunt-ended fragments have less GC bias as compared to fragments generated in the absence of said additive.

2. claims: 15-21

   a method of obtaining sequence information from a genome, said method comprising: (a) providing a population of first fragments of said genome; (b) preparing emulsion droplets of said first fragments, such that each emulsion droplet comprises a subset of said population of first fragments; (c) obtaining a population of second fragments within each emulsion droplet, such that said second fragments are shorter than said first fragments; (d) combining said emulsion droplets of said second fragments with emulsion droplets of adaptor tags; (e) ligating said second fragments with said adaptor tags to form tagged fragments; (f) combining said tagged fragments into a single mixture; (g) obtaining sequence reads from said tagged fragments, wherein
said sequence reads include sequence information from said adaptor tags and said fragments to identify fragments from the same emulsion droplet, thereby providing sequence information for said genome.

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<td>DK 1442134 T3</td>
<td>12-01-2009</td>
<td>EP 1442134 A2</td>
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