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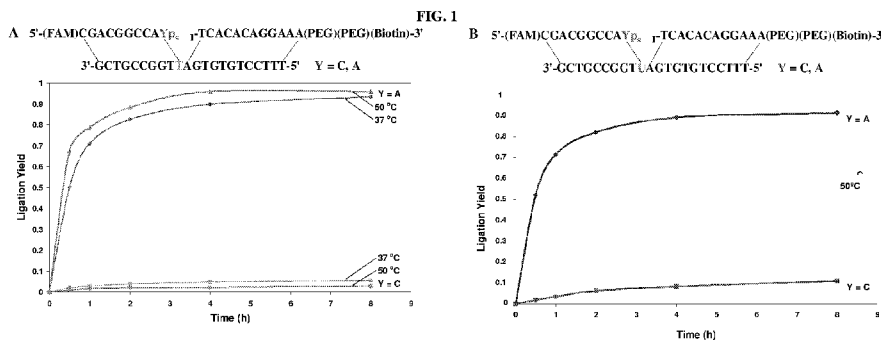
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(54) Title: CHEMICAL LIGATION



(57) Abstract: Methods comprising chemical ligation of oligonucleotides are provided. In some embodiments, methods of detecting a polymorphisms in nucleic acids are provided. In some embodiments, methods of detecting at least one analyte are provided. In some embodiments, methods of labeling solid support particles are provided. Kits comprising oligonucleotides with chemically ligatable moieties are also provided.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/032802

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12Q1/68 G01N33/48 G01N33/542
 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 G01N

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 | WO 2010/114599 A1 (DX TERITY DIAGNOSTICS INC [US]) 7 October 2010 (2010-10-07) paragraph [0042] - paragraph [0059]; claims 10,30,31; figure 1; examples 2,4 paragraph [00148] | 1-11, 46-53 |
| X | US 2006/160125 A1 (KOOL ERIC T [US]) 20 July 2006 (2006-07-20) | 1-8,11, 46-53 |
| Y | the whole document | 9,10 |
| X | WO 01/92579 A2 (PE CORP NY [US]; WENZ HANS MICHAEL [US]; SCHROTH GARY P [US] PE CORP N) 6 December 2001 (2001-12-06) cited in the application | 1-8,10, 11,46-51 |
| Y | page 4, line 23 - line 27 page 17, line 15 - page 21, line 3 page 13 - page 15; claims 1,17-19, 27, 42-44, 60, 64-69; example 1 | 9,52,53 |
| | ----- -/-- | |

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 | "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 |
| "E" earlier application or patent but published on or after the international filing date | "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 |
| "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) | "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 |
| "O" document referring to an oral disclosure, use, exhibition or other means | "&" document member of the same patent family |
| "P" document published prior to the international filing date but later than the priority date claimed | |

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| Date of the actual completion of the international search 25 July 2012 | Date of mailing of the international search report 02/11/2012 |
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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 Schmitt, Anja |
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/032802

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | US 2005/208503 A1 (YOWANTO HANDY [US] ET AL) 22 September 2005 (2005-09-22) cited in the application | 1-8,11, 46-49 |
| Y | the whole document | 9,10, 50-53 |
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| X | WO 2007/133703 A2 (DXTERITY DIAGNOSTICS [US]; TERBRUEGGEN ROBERT [US]) 22 November 2007 (2007-11-22) | 1,3,5,6, 8,9,11, 46-49 |
| Y | the whole document | 50-53 |
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| A | US 2002/064779 A1 (LANDEGREN ULF [SE] ET AL) 30 May 2002 (2002-05-30) cited in the application the whole document paragraph [0052]; claim 7 paragraph [0060] | 1-11 |
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INTERNATIONAL SEARCH REPORT

International application No.
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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, 50-53(completely); 46-49(partially)

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, 50-53(completely); 46-49(partially)

Methods of detecting a SNP in a target nucleic acid, comprising contacting said target nucleic acid with a first allele-specific primer that hybridizes to a portion of the target nucleic acid comprising the SNP and a locus-specific primer, wherein the first allele-specific primer comprises a 3' nucleophile, and the locus-specific primer comprises a 5' leaving group (or vice versa), wherein the first allele specific primer and the locus-specific primer hybridize to the target nucleic acid such that the 5' end of the locus-specific primer is adjacent to the 3' end of the first allele-specific primer, under conditions allowing chemical ligation between the first allele-specific primer and the first locus-specific primer to form a ligated product; and detecting the ligated product.

Kits comprising a first allele-specific primer and a locus-specific primer, wherein the first allele-specific primer comprises a 3' nucleophile, and the locus-specific primer comprises a 5' leaving group (or vice versa), wherein the first allele specific primer hybridizes to a portion of a target nucleic acid comprising a SNP, and wherein the first allele-specific primer and the locus-specific primer hybridize to a target nucleic acid such that the 5' end of the locus-specific primer is adjacent to the 3' end of the first allele-specific primer.

2. claims: 12-23, 54-65(completely); 46-49(partially)

A method of detecting at least one target analyte in a sample, comprising: (a) contacting the at least one target analyte with: (i) a first proximity detection probe comprising a first analyte binding moiety and a first oligonucleotide moiety, wherein the first oligonucleotide moiety comprises a 3' nucleophile; (ii) a second proximity detection probe comprising a second analyte binding moiety and a second oligonucleotide moiety, wherein the second oligonucleotide moiety comprises a 5' leaving group; and (iii) a splint oligonucleotide comprising a first portion that hybridizes with a portion of the first oligonucleotide moiety and a second portion that hybridizes with the second oligonucleotide moiety such that the 3' end of the first oligonucleotide moiety is adjacent to the 5' end of the second oligonucleotide moiety; under conditions allowing formation of a complex comprising at least one target analyte, the first proximity detection probe, the second proximity detection probe, and the splint oligonucleotide, and allowing chemical ligation between the first oligonucleotide moiety and the second oligonucleotide moiety to form a ligated product; and (b) detecting the ligated product.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A kit comprising a first proximity detection probe comprising a first analyte binding moiety and a first oligonucleotide moiety, wherein the first oligonucleotide moiety comprises a 3' nucleophile; and a second proximity detection probe comprising a second analyte binding moiety and a second oligonucleotide moiety, wherein the second oligonucleotide moiety comprises a 5' leaving group.

3. claims: 24-45(completely); 46-49(partially)

Methods of labeling a solid support particle, comprising contacting a solid support particle comprising a first member of a binding pair, with (i) a first oligonucleotide moiety comprising a 3' nucleophile, and further comprising a second member of a binding pair; (ii) a second oligonucleotide moiety comprising a 5' leaving group, and further comprising at least one detectable label; and (iii) a splint oligonucleotide comprising a first portion that hybridizes with a portion of the first oligonucleotide moiety and a second portion that hybridizes with the second oligonucleotide moiety such that the 3' end of the first oligonucleotide moiety is adjacent to the 5' end of the second oligonucleotide moiety; under conditions allowing binding of the first member of the binding pair to the second member of the binding pair, and allowing formation of a complex comprising the solid support particle, the first oligonucleotide moiety, the second oligonucleotide moiety, and the splint oligonucleotide, and allowing chemical ligation between the first oligonucleotide moiety and the second oligonucleotide moiety.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/032802

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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