



- (51) International Patent Classification:
C07H 21/04 (2006.01) C12Q 1/68 (2006.01)
- (21) International Application Number:
PCT/US2011/061701
- (22) International Filing Date:
21 November 2011 (21.11.2011)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/417,141 24 November 2010 (24.11.2010) US
- (71) Applicant (for all designated States except US): THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): VALLEE-BELISLE, Alexis [CA/US]; 935 Camino Pescadero B, Goleta, CA 93117 (US). RICCI, Francesco [IT/IT]; Via Giovanni Barracco, 12, I-00162 Rome (IT). WHITE, Ryan [US/US]; University of California, Santa Barbara, CA 93106 (US). BONHAM, Andrew J. [US/US]; University

of California, Santa Barbara, CA 93106 (US). PLAXCO, Kevin W. [US/US]; 2794 Williams Way, Santa Barbara, CA 93105 (US).

(74) Agent: NG, Rudy J.; 1900 University Avenue, Suite 200, East Palo Alto, CA 94303 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, 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.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

[Continued on next page]

(54) Title: NUCLEOTIDE-BASED PROBES AND METHODS FOR THE DETECTION AND QUANTIFICATION OF MACROMOLECULES AND OTHER ANALYTES

(57) Abstract: Provided are unimolecular oligonucleotide probes for detecting a target in a sample. The probes use target binding-induced structural changes to detect the presence of the target in the sample. Also provided are methods of using the probes to detect a target in a sample.

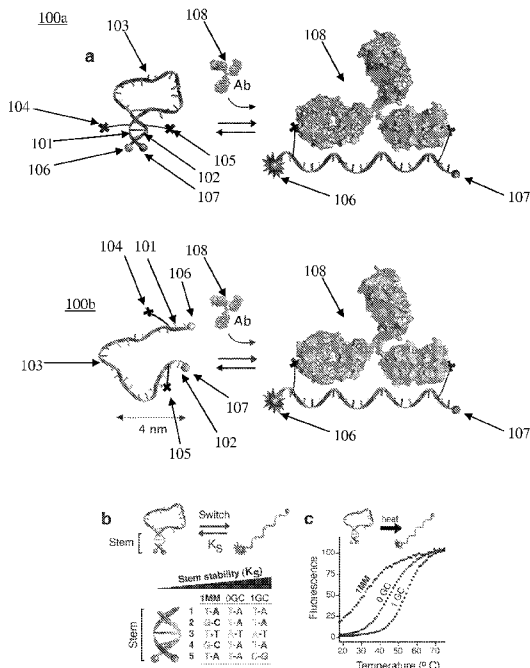
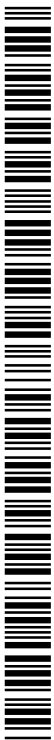


FIG. 1





SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

— *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))*

Published:

— *with international search report (Art. 21(3))*

(88) Date of publication of the international search report:

26 July 2012

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/61701

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C07H 21/04, C12Q 1/68 (2012.01) USPC - 536/24.3, 435/6.11 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) IPC(8) - C07H 21/04, C12Q 1/68 (2012.01) USPC - 536/24.3, 435/6.11 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 536/23.1, 435/6.1 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubWest (PGPB,USPT,USOC,EPAB,JPAB); PubMed (MEDLINE) detection, method, system, unimolecular, probe, beacon, protein, polypeptide, target, duplex, hybridization, sequence, binding, fluorophore, quencher, aptamer		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 5,925,517 A (TYAGI et al.) 20 July 1999 (20.07.1999) col 1, ln 10-12; col 3, ln 51 - col 4, ln 21; col 4, ln 47-48; col 4, ln 58-65; col 5, ln 65 - col 6, ln 3; col 6, ln 12-13; col 6, on 16-17; col 7, ln 47-67; col 9, ln 40; col 10, ln 66 - col 11, ln 1; col 11, ln 12-18	1-3, 9-10, 14, 21 ----- 4-8, 11-13, 15-20, 22-25
Y	WO 2010/048002 A1 (SOH et al.) 29 April 2010 (29.04.2010) para [0008], [0010], [0017], [0022], [0026], [0050], [0051], [0080], [0081], [0084], [0091], [0094]	4-8, 11-13, 18-20, 25
Y	US 2004/0005595 A1 (BROWNE) 08 January 2004 (08.01.2004) para [0044], [0045], [0050]	7, 8
Y	US 2007/0202498 A1 (AOJULA et al.) 30 August 2007 (30.08.2007) para [0009], [0010], [0020], [0038], [0086]; claim 20	15
Y	US 2009/0104614 A1 (TSOURKAS et al.) 23 April 2009 (23.04.2009) para [0027],[0093]	16, 17 ✓
A	US 2004/0219523 A1 (STANTON et al.) 04 November 2004 (04.11.2004) para [0009], [0154], [0279], [0330], [0718]	1-25
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* 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 08 May 2012 (08.05.2012)		Date of mailing of the international search report 30 MAY 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/61701

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:

This application contains the following inventions or groups of inventions which are not so linked to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I claims 1-25 directed to a system for detecting one or more targets in a sample.

Group II claims 26-46 directed to a system for detecting a DNA binding protein in a sample.

*** see continuation 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 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.:
Claims 1-25 (Group I)

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.

Continuation of: Box No. III Observations where unity of invention is lacking

The inventions listed as Groups I and II do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The special shared technical feature of Groups I and II is a unimolecular oligonucleotide probe configured to produce a detectable change in signal when contacted by one or more targets, the probe comprising a first hybridization sequence and a second hybridization sequence, wherein the first hybridization sequence and the second hybridization sequence are configured to form a duplex in the absence of the target binding to both the first target binding moiety and the second target binding moiety, such that the first target binding moiety is positioned adjacent the second target binding moiety, and a first signaling moiety and a second signaling moiety configured such that the position of the first signaling moiety is changed relative to the second signaling moiety upon binding of the one or more targets to both the first target binding moiety and the second target binding moiety, wherein in the presence of binding of the one or more targets to both the first target binding moiety and the second target binding moiety, formation of the duplex is inhibited such that the probe is configured to position the first signaling moiety relative to the second signaling moiety such that the probe produces a detectable change in signal. However, this is not an improvement over the prior art of US 5,925,517 A to Tyagi et al. that teaches a unimolecular oligonucleotide probe (col 4, ln 59-64) configured to produce a detectable change in signal when contacted by one or more targets (col 4 ln 59- col 5 ln 6; col 9 ln 36-42), the probe comprising a first hybridization sequence and a second hybridization sequence, wherein the first hybridization sequence and the second hybridization sequence are configured to form a duplex in the absence of the target binding to both the first target binding moiety and the second target binding moiety (col 4, ln 44-48; 'in the absence of target, interact with one another to form a stem duplex'), such that the first target binding moiety is positioned adjacent the second target binding moiety, and a first signaling moiety and a second signaling moiety configured such that the position of the first signaling moiety is changed relative to the second signaling moiety upon binding of the one or more targets to both the first target binding moiety and the second target binding moiety (col 11 ln 17-18) wherein in the presence of binding of the one or more targets to both the first target binding moiety and the second target binding moiety, formation of the duplex is inhibited such that the probe is configured to position the first signaling moiety relative to the second signaling moiety such that the probe produces a detectable change in signal (col 5 lns 13-43; col 6 ln 8-20). Group I differs specifically from Group II, because Group I does not require a recognition duplex specifically bound by a DNA binding protein in the sample; a first hybridization sequence and a second hybridization sequence, wherein the first and second hybridization sequences are configured to form a second duplex in the absence of binding of the DNA binding protein to the recognition duplex; nor a third hybridization sequence and a fourth hybridization sequence, wherein the third and fourth hybridization sequences are configured to form a third duplex in the absence of binding of the DNA binding protein to the recognition duplex.
Therefore the inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. In order for all inventions to be examined, the appropriate examination fees must be paid.