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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

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

(88) Date of publication of the international search report:
10 January 2013

(54) Title: MULTIFUNCTIONAL PROBE-PRIMERS

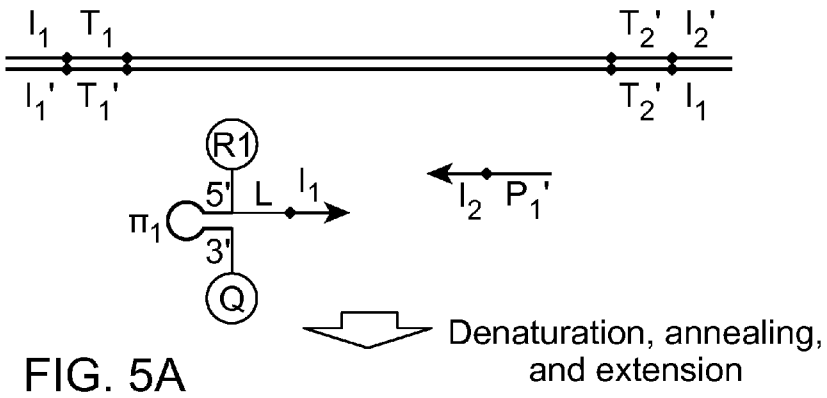

FIG. 5A

(57) Abstract: Methods and reagents for detection and analysis of nucleic acids are provided. Certain methods involves an encoding amplification in which a target sequence is associated with probe-binding sequences and optionally with indexing sequences, (2) an optional distribution step in which the product of the encoding amplification is split into multiple aliquots, and (3) a decoding and detection step in which the presence, absence, quantity, or relative amount of the target sequence in the aliquots is determined. The detection step makes use of a multifunctional "self-digesting" molecular probe comprising a primer polynucleotide and a probe oligonucleotide, linked in a 5'-5' orientation.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/23870

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C07H 21/00 (2012.01)
USPC - 435/6.12; 536/24.3
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)
USPC: 435/6.12; 536/24.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.11, 91.2; 536/24.33
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed
Search terms: 5', end, primer, probe, linker, linked, molecular beacon, fluorescent, non-nucleotide, orientation, inverted, reverse, multimer, bifunctional, hairpin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0003305 A1 (BRENTANO et al.) 6 January 2011 (06.01.2011) para [0026]; Fig. 1	1-9
Y	US 2010/0291557 A1 (LIVAK et al.) 18 November 2010 (18.11.2010) para [0068], [0119], [0145]; Fig. 4, 6	1-9
A	US 2004/0005595 A1 (BROWNE) 8 January 2004 (08.01.2004)	1-9
A	BROWNE. Sequence-specific, self-reporting hairpin inversion probes. J. Am. Chem. Soc. 16 February 2005 (16.02.2005), Vol. 127, No. 6, pages 1989-1994	1-9
A	MCKEEN et al. Synthesis of fluorophore and quencher monomers for use in scorpion primers and nucleic acid structural probes. Org. Biomol. Chem. 7 July 2003 (07.07.2003), Vol. 1, No. 13, pages 2267-2275	1-9

☐ Further documents are listed in the continuation of Box C. ☐

* 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	Date of mailing of the international search report
12 July 2012 (12.07.2012)	16 AUG 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

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/23870

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.: 10-13, 18-21, 29-30, 32-35, 39-48
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 as 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 1-9, directed to a multifunctional molecular probe, comprising: a) a first oligonucleotide that is a primer with an extendible 3' terminus; and b) a second oligonucleotide, comprising a first signal moiety; wherein the first oligonucleotide and the second oligonucleotide are connected by a linker in a 5'-5' orientation.

Group II: claims 14-17, 31 and 36-38 directed to a molecular construct comprising two polynucleotides and a non-nucleotide linker, wherein the first polynucleotide is an oligonucleotide comprising a signal moiety and comprising a sequence .pi.; the second polynucleotide comprises a target sequence and a probe binding sequence P, the target sequence being 5' to P; wherein .pi. and P are sufficiently complementary to each other to hybridize and form a double-stranded polynucleotide segment; and wherein the linker links the first and second polynucleotides in a 5'-5' orientation.
---please see continuation on extra 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.:
1-9

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/US 12/23870

Box No. III Observations where unity of invention is lacking

Group III: claims 22-28, directed to a paiir of molecular constructs, each comprising three linked polynucleotides, wherein 1) the first molecular construct comprises a first polynucleotide that is an oligonucleotide comprising a sequence .pi.sub.1 and a first signal moiety; a second polynucleotide that is an oligonucleotide comprising a sequence .pi.sub.2 and a second signal moiety; a third polynucleotide that comprises a first target sequence, and a probe binding sequence P.sub.1, the target sequence being 5' to P.sub1; and wherein 2) the second molecular construct comprises a second polynucleotide that is an oligonucleotide comprising a sequence .pi.sub.1 and the first signal moiety; a second polynucleotide that is an oligonucleotide comprising a sequence .pi.sub.2 and the second signal moiety; a third polynucleotide that comprises a second target sequence, and a probe binding sequence P.sub.2, the target sequence being 5' to P.sub.2; wherein, in the first molecular construct, P.sub.1 is sufficiently complementary to .pi.sub.1 to hybridize to form a double-stranded polynucleotide segment; and wherein, in the second molecular construct, P.sub.2 is sufficiently complementary to .pi.sub.2 to hybridize and form a double-stranded polynucleotide segment; and wherein the first and second target sequences are different; wherein the first and second signal moieties are different; wherein probe binding sequences P.sub.1 and P.sub.2 are different; and wherein, in each construct, a linker links the first polynucleotide and the third polynucleotide in a 5'-5' orientation.

The inventions listed as Groups I - III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the claims of Groups I-III are disclosed in the Group descriptions, above.

The only common technical element shared by all of the above groups is that they are related to oligonucleotides that are linked by a non-nucleotide linker in a 5'-5' orientation, wherein one of the two linked oligonucleotides is labeled. Groups II and III share the further common technical elements of a first polynucleotide comprising a target-binding sequence and a probe binding sequence P, wherein the linked probe comprises a sequence .pi. that is complementary to the probe-binding region of the first polynucleotide. These common technical elements do not represent an improvement over the combined prior art of US 2011/0003305 A1 to Brentano et al. (hereinafter "Brentano") in view of US 2010/0291557 A1 to Livak et al. (hereinafter "Livak"). In particular, Brentano teaches "a first amplification oligomer indirectly joined to a second amplification oligomer complex... In an aspect, the first and second amplification oligomers are joined using a non-nucleotide linker...the first amplification oligomer member and the second amplification oligomer member of the amplification oligomer complex are both non-promoter primers joined at their 5' ends using a non-nucleotide linker" (para [0026]). Although Brentano does not specifically recite wherein either oligomer may be labeled, nor wherein the oligomers may comprise regions which are complementary to each other, in a related disclosure, Livak teaches a nucleobase clamp that associates with a primer (A clamp may be any structure that: (i) conducts sequence-specific hybridization to the clamp-specific portion of a probe of a binary composition and (ii) can bear one or more labels...clamps include an oligonucleotides or nucleic acid analog, e.g. PNA. The probe and clamp may form either a duplex or triplex structure (FIG. 2), binding by Watson/Crick and other base-pairing interactions (Froehler, 1997; Rumney, 1995). The clamp has one or more covalently-attached labels; para [0119], see Fig. 6). It would have been obvious to a person skilled in the art to combine the teaching of Brentano regarding 5'-5' linked oligonucleotide primers with the clamped and labeled primer structure of Livak in order to assure the association of the clamp with the priming oligonucleotide in a one-to-one manner and in a desired orientation. Livak further teaches wherein, in another configuration, the target binding region of the second nucleic acid is 5' to the P region (Fig. 2, note the label present in the clamp in the same configuration in Fig. 4).

Therefore, the inventions of Groups I - III lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Claim 6 is objected to as written because the claim from which it depends, claim 1, does not contain an antecedent for "the third oligonucleotide". For the purpose of this search and opinion, claim 6 has been considered to depend from claim 5.