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(54) Title: SMALL NUCLEIC ACID DETECTION PROBES AND USES THEREOF

(57) Abstract: The present teachings are directed to compositions, methods, and kits for detecting and quantitating small nucleic acid molecules, including small DNA molecules and small RNA molecules. The detector probes of the current teachings, including single-loop detector probes, double-loop detector probes, and bimolecular detector probes, are designed to selectively hybridize with a corresponding small nucleic acid target and to produce, under appropriate conditions, a detectable signal or a detectably different signal. The detector complexes of the current teachings comprise a detector probe comprising a first reporter group and a displaceable sequence comprising a second reporter group, wherein the displaceable sequence is hybridized to the detector probe. According to certain methods, detecting a small nucleic acid target comprises the target displacing the displaceable sequence of a detector complex to form a detector probe-small nucleic acid target duplex, illuminating the duplex with light of an appropriate wavelength, and determining the presence of a detectable fluorescent signal or the change in a detectable signal.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/05828

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12Q 1/68**(2006.01);**C07H 21/02**(2006.01)

USPC: 435/6;536/23.1
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)
U.S. : 435/6; 536/23.1

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)
US Patent Database, US-PGPUB Database

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 6,468,808 B1 (NIE et al) 22 October 2002 (22.10.2002), figure 1.	1 --- 5, 6, 38, 39
X --- Y	US 2003/0165912 A1 (SORGE et al) 04 September 2003 (04.09.2003), figure 2E, 2G, paragraphs [0027], [0028], [0030], [0044], [00447], [0063]	1, 2 --- 3-6, 38, 39
Y	KUTYAVIN et al. 3'-Minor groove binder-DNA probes increase sequence specificity at PCR extension temperatures. Nucleic Acids Research (2000) vol 28, no 2, pp 655-661. See entire document.	3-6, 38, 39
A	US 2003/0165935 A1 (VANN et al) 04 September 2003 (04.09.2003), figure 1, paragraph [0004]	1-6 and 38-39

☒ 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

"E" earlier application or patent 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
24 May 2007 (24.05.2007)	04 SEP 2007
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 Samuel Woolwine Telephone No. (571) 272-1600

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003/0143535 A1 (LYAMICHEV et al) 31 July 2003 (31.07.2003), figure 11A, paragraphs [0508]-[0513]	1-6 and 38-39

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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:
Please 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 any 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.: 1-6,38 and 39

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/US06/05828

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

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, claim(s) 1-6, 38 and 39, drawn to stem loop detector probes.

Group II, claim(s) 7, 40 and 41, drawn to bimolecular detector probes.

Group III, claim(s) 8, 9 and 35-37, drawn to stem loop detector probes in combination with a displaceable sequence.

Group IV, claim(s) 10-16, drawn to methods for detecting nucleic acid sequences using detection probes comprising displaceable sequences.

Group V, claim(s) 17-28, drawn to methods for detecting nucleic acid sequences using stem loop detection probes.

Group VI, claim(s) 29-34, drawn to methods for detecting nucleic acid sequences using bimolecular detection probes.

The inventions listed as Groups I-VI 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 groups listed above differ in the types of probe (stem loop, bimolecular, or stem loop with displaceable sequence). The only feature shared by all groups is the concept of a detection probe for detecting nucleic acids. Barring this general feature, the groups may be linked in pairs as a product and method of using that product as follows: Groups I and V, Groups II and VI, Groups III and IV. However, Nie et al (USPN 6,468,808) teach a detection probe comprising a stem loop meeting the limitations of claim 1 (see figure 1, where the "Quantum dot" serves as a reporter group). This teaching anticipates claim 1 and the general concept of using a detector probe to detect a nucleic acid sequence (breaking unity among the pairs of groups described above). Kutyavin et al (Nucleic Acids Research, 28(2): 655-661 (2000)) teach tethering intercalating dyes to nucleic acid probes to increase specificity. Taken together, Nie et al and Kutyavin et al render obvious at least claim 38 (breaking unity between Group I and V). Van et al (US 2003/0165935 A1) teach a bimolecular detector probe complex anticipating claims 7 and 29 (see figure 1 and paragraph [0004], for example), which breaks unity of invention between Groups II and VI. Finally, Lyamichev et al (US 2003/0143535 A1) teach a complex meeting the structural limitations of the "detector complex" of claim 8 (see bottom half of figure 11A and paragraphs [0508]-[0513]; the stem loop structure (#80) is labeled with fluorescein as a reporter group, and the displaceable sequence (any of #78, 4, 79, or 116) are labeled with biotin as a reporter group...biotin is consistent with Applicant's description of reporter groups at page 8, paragraph [0021]). The Lyamichev et al disclosure breaks unity of invention between Groups III and IV.