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- (71) Applicant: **QTL BIOSYSTEMS, LLC** [US/US]; 2778 Agua Fria Street, Building C, Suite BC, Santa Fe, NM 87507 (US).
- (72) Inventors: **KUMARASWAMY, Sriram**; 2500 Sawmill Road, Apt. #312, Santa Fe, NM 87505 (US). **WHITTEN, David**; 811 D West Manhattan Avenue, Santa Fe, NM 87501 (US). **MCBRANCH, Duncan**; 14 North Sparrow Lane, Santa Fe, NM 87501 (US). **RININSLAND, Frauke**; 2556 Camino San Patricio, Santa Fe, NM 87505 (US). **BURDICK, Brent, Arthur**; 31 Las Huertas Road, Placitas, NM 87043 (US).
- (74) Agents: **KELBER, Steven, B.** et al.; Piper Rudnick LLP, 1200 Nineteenth Street, N.W., Washington, DC 20036 (US).
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(54) Title: BIO-SENSING PLATFORMS FOR DETECTION AND QUANTITATION OF BIOLOGICAL MOLECULES

(57) Abstract: A bioconjugate comprising a fluorescer (P) linked to a quencher (Q) by a tether (T) is provided. The tether (T) includes a segment that can recognize and interact with a target biomolecule. In the absence of a specific interaction of the bioconjugate with an enzyme or other target biomolecule recognizing the bioconjugate, the fluorescence of the polymer (P) is attenuated or modified by the relatively close proximity thereto of the quencher (Q). As a consequence of the association of the bioconjugate with the target biomolecule, a reaction can occur which results in a cleavage of the bioconjugate tether and a release of the fluorescent polymer and/or quencher. This sequence of events can be followed by an enhancement or amplification of the polymer fluorescence.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/26697

A. CLASSIFICATION OF SUBJECT MATTER				
IPC(7) : G01N 33/53 US CL : 435/7.5				
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. : 435/7.5; 5,6,91.1; 91.2				
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)				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
Y	US 5,723,591 A (LIVAK et al.) 03 March 1998, Abstract and claims 1-24;	1-14, 50-55		
Y	LEE et al. Allelic discrimination by nick-translation PCR with fluorogenic probes. Nucleic Acids Research. 1993, Vol. 21, pages 3761-3766; Abstract, Figures 1-2 and	1-14, 15-31, 40-49		
Y	US 5,876,930 S (LIVAK et al.) 02 March 1999, Column 3-4; claims 1-15	32-39		
A	US 6,210,896 A (CCHAN) 03 April 2001, Column 14, claims 1-12	45-49		
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.				
* Special categories of cited documents: <table border="0" style="width:100%"> <tr> <td style="width:50%"> "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 </td> <td style="width:50%"> "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 </td> </tr> </table>			"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
"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		
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer <i>Hayne B. Redden</i> Long Le Telephone No. (703)308-0196		

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/26697

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim 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. Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 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 an additional fee, this Authority did not invite payment of any additional fee.
 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.
- No protest accompanied the payment of additional search fees.

BOX II. 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) 1-14 and 52-55, drawn to a bioconjugate comprising a segment capable of recognizing and interacting with the target molecule comprising a sequence selected from the group consisting of: a peptide; a nucleic acid, an oligosaccharid; and a glycoconjuate.

Group II, claim(s) 15-31, drawn to a method of assaying for the presence of a target in a sample, comprising the "special technical feature" of the step providing a segment capable of recognizing and interacting with the target molecule in between a fluorescer and a quencher molecules.

Group III, claim(s) 32-39, drawn to a method of assaying for the presence of a target analyte in a sample, comprising the "special technical feature" of the step incubating the sample with a bioconjugate comprising a quencher and a reactive group conjugated to a tether at first and second locations, respectively.

Group IV, claim(s) 40-44, drawn to an intracellular assay for analyte, comprising the "special technical feature" of the step transfecting a cell with a bioconjugate as set forth in Group I.

Group V, claim(s) 45-49, the first product used in Group I, drawn to a biotinylated fluorescer.

Group VI, claim(s) 50-51, the second product used in Group I, drawn to a quenching reagent.

The invention listed as Groups I-VI do not relate to a single 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:

Group I recites a bioconjuate composition capable of recognizing and interacting the target molecule and providing the fluorescence-quenching to detect the presence of the target molecule. Both Livak et al. (USP 5723591) and Lee et al. (Nucleic Acids Research 21 (1993): 3761-3766) teach the similar method using a probe comprising a fluorescent indicator dye and a quencher to detect the change of fluorescence signal due to the hybridization of the probe with the target polynucleotides. Therefore, the invention of Group I does not fulfill the requirement for unity of invention under Rule 13.1 PCT. Accordingly, the different products used in Group I, such as Groups V and VI and the method Group II, III and V using Group I conjugate are, are not so linked as to form a single general inventive concept.

As set forth above, each method of Group II, III and IV, has special technical feature not shared by the other Groups. Therefore, the inventions listed as Groups II-IV do not relate to a single general inventive concept because the lack of the same or corresponding technical feature.