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(54) Title: MULTIPLE REPORTER READ-OUT FOR BIOASSAYS

(57) Abstract: A method for detecting a plurality of reactive sites on an analyte, comprising allowing reactants on an addressable microsphere and the reactive sites to react, forming reactant-reactive site pairs distinguishable by fluorescence intensity. The invention also provides a method for detecting a plurality of analytes in a sample using addressable microspheres in combination with one or more reporter reagents. Also provided are a method for determining allele zygosity of a genetic locus having two alleles or more alleles using microparticles, and a method for detecting a plurality of SNPs in nucleic acid molecules. The instant invention also provides a composition comprising an addressable microsphere carrying at least two fluorescent reactants capable of forming reactant-analyte pairs distinguishable by their fluorescence intensity, and kits comprising the inventive composition and a plurality of reporter reagents.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/29743A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

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 7 C12Q

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 practical, search terms used)

INSPEC, EPO-Internal, WPI Data, BIOSIS, MEDLINE, EMBASE, LIFESCIENCES, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FULTON R J ET AL: "Advanced multiplexed analysis with the FlowMetrix(TM) system" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 43, no. 9, September 1997 (1997-09), pages 1749-1756, XP002142645 ISSN: 0009-9147 the whole document ----- -/--	1-27

 Further documents are listed in the continuation of box C. Patent family members are listed in 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 document 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

30 December 2002

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INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/US 01/29743

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SMITH P L ET AL: "A RAPID, SENSITIVE, MULTIPLEXED ASSAY FOR DETECTION OF VIRAL NUCLEIC ACIDS USING THE FLOW METRIX SYSTEM" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 44, no. 9, 1998, pages 2054-2056, XP002170109 ISSN: 0009-9147 page 2055, left-hand column, paragraph 3 -page 2056, left-hand column, paragraph 2 ---	1-27
X	US 5 981 180 A (CHANDLER MARK B ET AL) 9 November 1999 (1999-11-09) column 1, line 45 -column 6, line 39 column 18, line 33 -column 26, line 29 ---	1-27
X	US 5 736 330 A (FULTON R JERROLD) 7 April 1998 (1998-04-07) column 1 -column 7, paragraph 1; figures 1,2 ---	1-10, 13-17, 20-27
X	TYAGI S ET AL: "Multicolor molecular beacons for allele discrimination" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 16, 1998, pages 49-53, XP002143901 ISSN: 1087-0156 abstract page 49, right-hand column -page 52, right-hand column, paragraph 3 ---	1-10, 13-17, 20-27
A	KETTMAN J R ET AL: "CLASSIFICATION AND PROPERTIES OF 64 MULTIPLEXED MICROSPHERE SETS" CYTOMETRY, ALAN R. LISS, INC, XX, vol. 33, no. 2, 1 October 1998 (1998-10-01), pages 234-243, XP001041450 ISSN: 0196-4763 abstract page 241, left-hand column, paragraph 2 -page 242, left-hand column -----	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/29743

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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:
see FURTHER INFORMATION sheet PCT/ISA/210

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 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:

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 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.:
8-10 all completely, 1-7 and 17 all partially

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-7, 17 and 25-27 relate to an extremely large number of possible methods and compounds. In fact, the claims contain so many options, that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely the methods as set out in examples 1 to 7 (location of polymorphisms or mutations in example 1, detection of sequence variations in the BCRA1 gene in example 3, measuring of human chorionic gonadotropin variants in example 5).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 8-10 all completely, 1-7 and 17 all partially

A method for the detection of one or more alleles of a locus on a nucleic acid molecule providing a population of microspheres, wherein each of the microspheres carries a plurality of fluorescently-labelled nucleic acid probes respectively specific for the one or more alleles and allowing the one or more alleles to react with the labelled nucleic acid probes, thereby forming reactant-reactive site pairs which are detectably distinguishable from each other and detecting said site pairs.

2. Claims: 11, 12

A method for the detection of one or more epitopes of an antigen molecule providing a population of microspheres, wherein each of the microspheres carries a plurality of fluorescently-labelled antibodies respectively specific for the one or more epitopes and allowing the antigen to react with the labelled antibodies, thereby forming reactant-reactive site pairs which are detectably distinguishable from each other and detecting said site pairs.

3. Claims: 13-16

A method for determining of allele zygosity of a nucleic acid molecule on a genetic locus providing a population of microspheres, wherein each of the microspheres carries two nucleic acid probes respectively specific for each of the two alleles and allowing the probes to hybridise to the alleles, thereby forming allele-probe pairs which are detectably distinguishable from each other and detecting the presence or absence and fluorescence intensity of the allele-probe pairs, whereby the allele zygosity of the locus is determined.

4. Claims: 17-24

A method for detecting a plurality of analytes in a sample, the method comprising (1) providing a population of microspheres, wherein each microsphere carries a reactant capable of reacting respectively to each of the plurality of analytes; (2) allowing the reactant and the analytes to react, thereby forming reactant-analyte pairs; (3) providing a mixture of a plurality of reporter reagents capable of reacting with the reactant-analyte pairs; (4) allowing the reporter reagents to react with the reactant-analyte pairs to form reactant-analyte-reporter reagent complexes which

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

are distinguishable by fluorescence intensity; and (5) detecting the presence or absence of the reactant-analyte-reporter reagent complexes formed, whereby the presence or absence of each of the plurality of analytes is determined.

5. Claims: 25-27

A composition comprising an addressable microsphere

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/29743

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5981180	A	09-11-1999	AU	7398996 A	30-04-1997
			CA	2227895 A1	17-04-1997
			EP	0852004 A2	08-07-1998
			WO	9714028 A2	17-04-1997
			US	6524793 B1	25-02-2003

US 5736330	A	07-04-1998	AU	7398996 A	30-04-1997
			CA	2227895 A1	17-04-1997
			EP	0852004 A2	08-07-1998
			US	6057107 A	02-05-2000
			WO	9714028 A2	17-04-1997
			US	6524793 B1	25-02-2003
