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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD AND COMPOSITIONS FOR DETECTION AND ENUMERATION OF GENETIC VARIATIONS

(57) Abstract: Many areas of biomedical research depend on the analysis of uncommon variations in individual genes or transcripts. Here we describe a method that can quantify such variation at a scale and ease heretofore unattainable. Each DNA molecule in a collection of such molecules is converted into a single particle to which thousands of copies of DNA identical in sequence to the original are bound. This population of beads then corresponds to a one-to-one representation of the starting DNA molecules. Variation within the original population of DNA molecules can then be simply assessed by counting fluorescently-labeled particles via flow cytometry. Millions of individual DNA molecules can be assessed in this fashion with standard laboratory equipment. Moreover, specific variants can be isolated by flow sorting and employed for further experimentation. This approach can be used for the identification and quantification of rare mutations as well as to study variations in gene sequences or transcripts in specific populations or tissues.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/15587

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(7) : C07H 21/04; C12Q 1/68
 US CL : 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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 392 546 A2 (DRMANAC et al.) 12 April 1990 (12.04.1990), see entire document.	1-6, 13-15
X	GHOSH et al. Covalent attachment of oligonucleotides to solid supports. Nucleic Acids Research. 1987, Vol. 15, No 13, pages 5353-5372, see entire document.	1-6, 13-15
X, P	US 2003/0129640 A1 (SASAKI et al.) 10 July 2003 (10.07.2003), see entire document.	1-16
X, P	US 2004/0185484 A1 (COSTA et al) 23 September 2004 (23.09.2004), see entire document, especially para 94-105.	35-54
Y, P	US 6,808,882 B2 (GRIFFITHS et al) 26 October 2004 (26.10.2004), see entire document.	35-54
Y	WO 00/40712 A1 (GRIFFITHS et al.) 13 July 2000 (13.07.2000), see entire document.	35-54

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.

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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) 1-34, drawn to a composition comprising a plurality of beads wherein the beads comprise polynucleotides.

Group II, claim(s) 35-54, drawn to a method for analyzing nucleotide sequence variations by forming microemulsions.

Group III, claim(s) 55-58, drawn to probes and pairs of probes.

Group IV, claim(s) 59-63, drawn to a method for isolating nucleotide sequence variants.

The inventions listed as Groups I-IV 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 prior art teaches a composition comprising a plurality of beads where the beads comprise polynucleotides, therefore, this is not a contribution over the art.

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material

a sequence listing

table(s) related to the sequence listing

b. format of material

in written format

in computer readable form

c. time of filing/furnishing

contained in the international application as filed

filed together with the international application in computer readable form

furnished subsequently to this Authority for the purposes of search

2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

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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 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.: 1-54

 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.