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(54) Title: METHOD FOR AMPLIFICATION OF MOLECULAR BIO-ASSAY SIGNALS

(57) Abstract: Disclosed are reagents and a method for effective *in vitro* amplification of bio-assay signals. The method makes use of a pair of "end-to-end" complementary oligonucleotide primers to continuously form a double-stranded and highly repetitive hybrid molecule. Since this hybrid molecule is covalently linked and added to the probing molecule in the bioassays, and is also compatibly labeled, it can amplify the detection signals hundreds of times within a short period. The method can have very broad applications in bioassays using nucleic acid hybridization, immunochemical detection, and other specific interactions between two molecules or between molecules and cells.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/38104

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12Q 1/68; C12P 19/34; C07H 21/04  
 US CL : 435/6, 91.1, 91/51; 536/23.1, 23.5, 23.6, 23.7, 24.3, 24.33

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, 91.1, 91/51; 536/23.1, 23.5, 23.6, 23.7, 24.3, 24.33

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)  
 Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIZARDI et al. Mutation Detection and Single-molecul Counting Using Isothermal Rolling-Circle Amplification. Nature Genetics. July 1998, Vol. 19, pages 225-232, see whole document.	1-32
A	SCHWEITZER et al. Immunoassays with Rolling Circle DNA amplification: A Versatile Platform for Ultrasensitive Antigen Detection. PNAS. 29 Augus 2000, Vol. 97, No. 18, pages 10113-10119, see whole document.	1-32
A	US 6,316,229 B1 (LIZARDI et al.) 13 November 2001 (13.11.2001), see whole document.	1-32

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	

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**INTERNATIONAL SEARCH REPORT**

PCT/US02/38104

**Continuation of B. FIELDS SEARCHED Item 3:**

Database: USPAT, PGPUB, JPO, EPO, Derwent, Medline, Biosis, CaPlus, Embase, Biotechds

Search Terms: primer, probe, covalent, amplifier, target-probe, symmetrical, reference primer, end-to end, rolling circle, amplification