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[Continued on next page]

(54) Title: AMPLIFICATION OF POLYNUCLEOTIDES BY ROLLING CIRCLE AMPLIFICATION

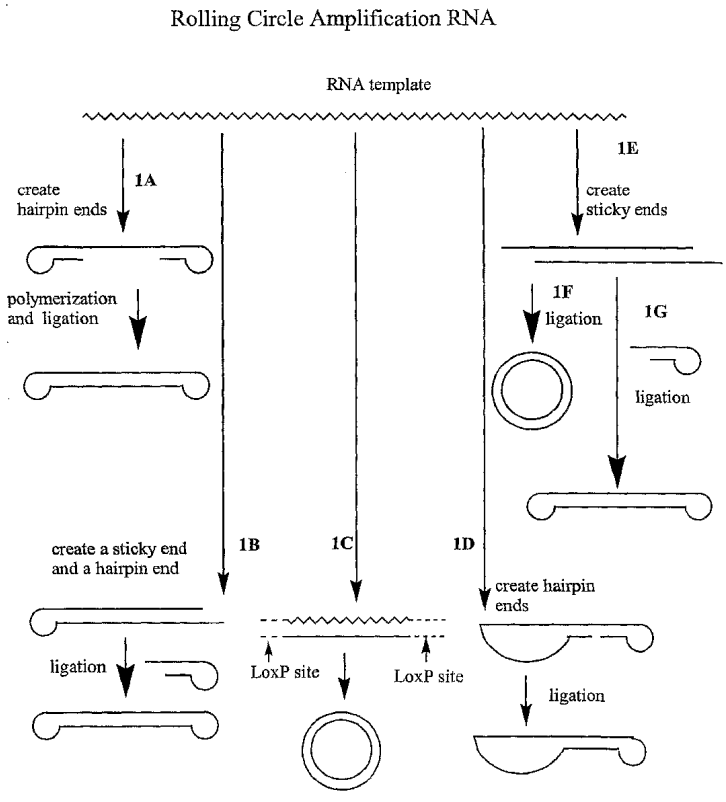

Figure 1

(57) Abstract: Rolling circle amplification is used to amplify and detect target nucleic acid molecules by affixing a first and/or second linker nucleic acid molecule or a second linker nucleic acid molecule to the target nucleic acid molecule, then circularizing the target nucleic acid molecule, and then amplifying the circularized nucleic acid molecule to generate reiterated nucleic acid sequences. The methods may be used to amplify nucleic acids, particularly RNA, for detection and cloning.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12Q 1/68**(2006.01);**C12P 19/34**(2006.01)

USPC: 435/6,91.2
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. : 435/6,91.2

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)
WEST (patent), pubmed, STN (caplus, medline biosis)

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 6,498,023 (ABARZUA et al.) 24 December 2002	1-16, 31-39, 54, 61, 70-82 --- 62, 64-69
Y	US 6,465,219 (ZHU et al.) October 2002	62, 64-69
Y	US 4,555,486 (BAHL et al.), November 1985	62, 64-69

☐ 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

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/31652

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.: 1-16,31-39,54 and 61-82

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.

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) 1-16, 31-39, 54, 61-82, drawn to a method of amplifying and detecting nucleic acids comprising affixing a linker, circularizing and amplifying the circularized target.

Group II, claim(s) 17-30, 40-53, 55-60 and 83-109, drawn to a method of amplifying nucleic acids comprising adding a circular nucleic acid probe to a target, hybridizing and generating nucleic acids complementary to the circular probe.

The inventions listed as Groups I-II 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 inventions listed as Groups I-II 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 inventions are not linked by a corresponding special technical feature that distinguishes the invention over the prior art. The method of group I has been taught by Abarzua et al. (US Patent 6,498,023; December 2003) in the prior art. Abarzua teaches the method as follows. Abarzua teaches a method of amplifying a polynucleotide, comprising: a) affixing a first oligonucleotide linker comprising a hairpin to one end of the target (Figure 4, where the hairpin ends of the linear target are ligated to form a circularized polynucleotide; col. 12, lines 13-35, where hairpin oligonucleotides can be ligated to the ends of DNA fragments of any size; see also col. 12, lines 43-61, where the linear segment can be generated in a manner other than through restriction nuclease digestion); b) ligating 3' and 5' ends of the linear target to form a circularized polynucleotide (Figure 4, where the hairpin ends of the linear target are ligated to form a circularized polynucleotide; col. 12, lines 13-35, where the fragments and hairpins are used to form circular DNA); and c) amplifying the circularized polynucleotide (col. 12, lines 21-35, where the circular polynucleotide is amplified using rolling circle amplification).