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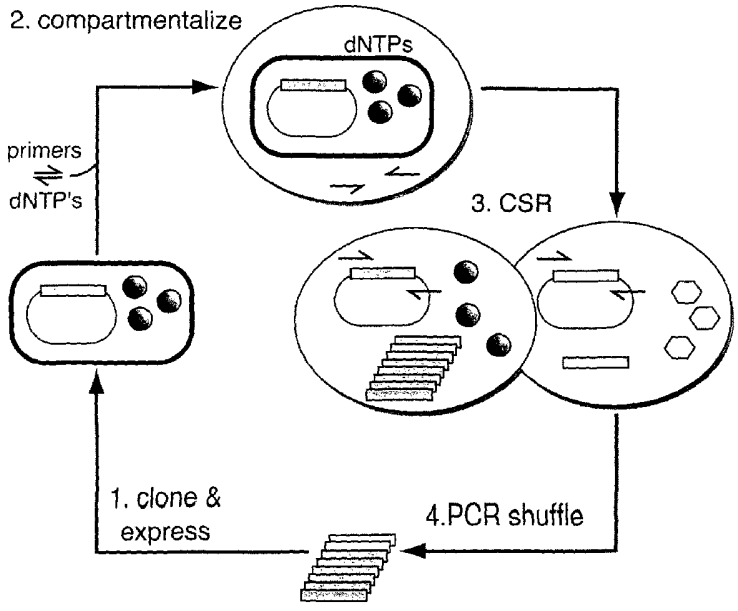
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[Continued on next page]

(54) Title: DIRECTED EVOLUTION METHOD



(57) Abstract: We describe a method of selecting an enzyme having replicase activity, the method comprising the steps of: (a) providing a pool of nucleic acids comprising members each encoding a replicase or a variant of the replicase; (b) subdividing the pool of nucleic acids into compartments, such that each compartment comprises a nucleic acid member of the pool together with the replicase or variant encoded by the nucleic acid member; (c) allowing nucleic acid replication to occur; and (d) detecting amplification of the nucleic acid member by the replicase. Methods for selecting agents capable of modulating replicase activity, and for selecting interacting polypeptides are also disclosed.

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Declaration under Rule 4.17:
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Published:
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/04108

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12N15/54 C12N9/12 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 C12N 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)
WPI Data, PAJ, CAB Data, SEQUENCE SEARCH, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GHADESSY FARID J ET AL: "Directed evolution of polymerase function by compartmentalized self-replication." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 98, no. 8, 10 April 2001 (2001-04-10), pages 4552-4557, XP002189673 April 10, 2001 ISSN: 0027-8424 cited in the application the whole document ---	1-7, 12-30
X	WO 99 02671 A (MEDICAL RES COUNCIL ;TAWFIK DAN (GB); GRIFFITHS ANDREW (GB)) 21 January 1999 (1999-01-21) cited in the application the whole document ---	1,2

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[X] Further documents are listed in the continuation of box C.

[X] 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

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"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
8 July 2002	24 07 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hornig, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 96 40723 A (GEN HOSPITAL CORP) 19 December 1996 (1996-12-19) the whole document	1-9, 12-30
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	SUZUKI M ET AL: "RANDOM MUTAGENESIS OF THERMUS AQUATICUS DNA POLYMERASE I: CONCORDANCE OF IMMUTABLE SITES IN VIVO WITH THE CRYSTAL STRUCTURE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, 1 September 1996 (1996-09-01), pages 9670-9675, XP002062865 ISSN: 0027-8424 cited in the application page 9671, right-hand column, paragraph 3 -page 9672, left-hand column, line 35 ---	1
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 01/04108

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:

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

As a result of the prior review under R. 40.2(e) PCT, no additional fees are to be refunded.

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.:

Claims: (1-9)-complete, (12-30)-partially corresponding to Inventions 1 and 2;

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. Claims: (1-7)-complete, (12-30)-partially

A method of selecting a nucleic acid processing (NAP) enzyme, the method comprising the step of: (a) providing a pool of nucleic acids comprising members encoding a NAP enzyme or a variant of the NAP enzyme; (b) subdividing the pool of nucleic acids into compartments, such that each compartment comprises a nucleic acid member of the pool together with the nAP enzyme or variant encoded by the nucleic acid member; (c) allowing nucleic acid processing to occur; and (d) detecting processing of the nucleic acid member by the NAP enzyme;

2. Claims: (8,9)-complete, (12-30)-partially

A method of selecting a pair of polypeptides capable of stable interaction, the method comprising: (a) providing a first nucleic acid encoding a first fusion protein comprising a first subdoain of a NAP enzyme fused to a first polypeptide, the second nucleic acid encoding a second fusion protein comprising a second subdomain of a NAP enzyme fused to a second polypeptide; in which stable interaction of the first and second NAP subdomains generates processing activity; and in which at least on of the first and second nucleic acids is provided in the form of a pool of nucleic acids encoding variants of the respective first and/or second polypeptide(s); (b) subdividing the pools of nucleic acids into compartments, such that each compartment comprises a first nucleic acid and a second nucleic acid together with respective fusion proteins encoded by the first and second nucleic acids; (c) allowing the first polypeptide to bind to the second polypeptide, such that binding of the first and second polypeptided leads to stable interaction of the NAP subdomains to generate NAP enzyme activity; and (d) detecting processing of at least one of the first and second nucleic acids by the NAP enzyme;

3. Claims: (10,11)-complete, (12-30)-partially

A method of selecting a polypeptide capable of stable folding, the method comprises: (a) providing a pool of nucleic acids comprising members encoding one or more candidate polypeptides fused to a NAP enzyme; (b) subdividing the pool of nucleic acids into compartments, such that each compartment comprises a nucleic acid member of the pool, the fusion polypeptide encoded by the nucleic acid member, and the NAP enzyme; and allowing for folding of the fusion polypeptide such that folded fusion prolypeptide will not inhibit NAP activity; and (c) detecting processing of the nucleic acid member by the NAP enzyme;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

4. Claims: 31-36,38-43

A NAP enzyme; said enzyme which has a greater thermostability than the corresponding unselected gene, said enzyme which is a Taq polymerase having more than 10 times increased half-life at 97.5°C when compared to wild type Taq polymerase; said Taq polymerase mutant comprising the mutations: F73S, R205K, K219E, M236T, E434D and A608V;

5. Claim : 37

Taq polymerase mutant comprising the mutations: K225E; E388V, K540R, D578G, N583S and M747R;

6. Claim : 44

Taq polymerase mutant comprising the mutations: G84A, D144G, K314R, E520G, check F598, A608V, E742G;

7. Claim : 45

Taq polymerase mutant comprising the mutations: D58G, R74P, A109T, L245R, R343G, G370D, E520G, N383S, E694K and A743P;

8. Claim : 46

A water-in-oil emulsion obtainable by emulsifying an aquaous phase with an oil phase in the presecnce of a surfactant comprising 4.5% v/v Span80, 0,4% v/v Tween80 and 0,1% v/v TritonX100, or a surfactant comprising Span80, Tween80 and TritonX100 in substantially the same proportions;

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 01/04108

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 01/04108

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