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

(54) Title: A METHOD FOR INCREASING THE PROCESSIVITY OF A DNA- OR RNA-DEPENDENT POLYMERASE AND COMPOSITIONS THEREFOR

(57) Abstract: The present invention relates to genetic engineering, and especially to cDNA synthesis and cDNA cloning. More specifically, a method is presented for increasing the processivity of a DNA- or RNA-dependent RNA- or DNA-polymerase comprising an addition of a general nucleic acid binding protein. In particular, the present invention relates to methods for increasing the processivity of reverse transcriptase (RT) *E. coli* DNA polymerase and T7 DNA polymerase using a nucleic acid binding protein such as Ncp7, recA, SSB and T4gp32. The invention further relates to assays to identify and select agents capable of increasing the processivity of a DNA or RNA-dependent polymerase, such as MMTV RT, AMV RT, T7 DNA polymerase and *E. coli* DNA polymerase. In a particularly preferred embodiment, the invention relates to a method for increasing the generation of full-length cDNA clones using a nucleic acid binding protein such as Ncp7, recA, SSB and T4gp32.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00261

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/12 C12N15/10

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

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)

EPO-Internal, WPI Data, PAJ, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LI X ET AL: "Human immunodeficiency virus Type 1 nucleocapsid protein (NCp7) directs specific initiation of minus-strand DNA synthesis primed by human tRNA(Lys3) in vitro: studies of viral RNA molecules mutated in regions that flank the primer binding site" JOURNAL OF VIROLOGY, US, THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 70, no. 8, 1 August 1996 (1996-08-01), pages 4996-5004, XP002088584 ISSN: 0022-538X page 4997, column 1, line 6 - line 25 page 4997, line 40 - line 63 page 5002, column 1, last paragraph -page 5002, paragraph 2 --- -/--	1,2,4-6, 8-11, 18-20,30

☒ 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

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"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

1 February 2001

Date of mailing of the international search report

15.3.01

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INTERNATIONAL SEARCH REPORT

Intr ional Application No

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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.
X	US 4 331 589 A (HUNG PAUL P ET AL) 25 May 1982 (1982-05-25) the whole document	1,2,6, 10-12, 21,30
A	---	4,5,8,9, 20
X	JI, X. ET AL.: "Effect of human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein on HIV-1 reverse transcriptase activity in vitro" BIOCHEMISTRY., vol. 35, 1996, pages 132-142, XP000946067 AMERICAN CHEMICAL SOCIETY. EASTON, PA., US ISSN: 0006-2960 abstract page 135, column 2, last paragraph -page 139, column 2, paragraph 3 ---	1-12,15, 18-21,30
X	RODRIQUEZ-RODRIQUEZ, L. ET AL.: "Influence of human immunodeficiency virus nucleocapsid protein on synthesis and strand transfer by the reverse transcriptase in vitro" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 270, 1995, pages 15005-15011, XP000946053 AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD., US ISSN: 0021-9258 abstract page 15007, paragraph 3 -page 15009, paragraph 2 ---	1-12,15, 18-21,30
X	LENER, D. ET AL.: "Involvement of HIV-1 nucleocapsid protein in the recruitment of reverse transcriptase into nucleoprotein complexes formed in vitro" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 273, 1998, pages 33781-33786, XP000946066 AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD., US ISSN: 0021-9258 abstract page 33784, column 2, paragraph 2 -page 33785, column 1, paragraph 2 page 33786, column 1, last paragraph -column 2, last paragraph ---	1-12,15, 18-21,30
A	EP 0 821 059 A (RIKAGAKU KENKYUSHO) 28 January 1998 (1998-01-28) page 4, line 7 - line 51 ---	1-3
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INTERNATIONAL SEARCH REPORT

International Application No

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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.
X	WO 91 17267 A (STANFORD RES INST INT) 14 November 1991 (1991-11-14) abstract page 4, line 5 -page 5, line 22 page 11, line 1 -page 18, line 25 ---	22-25, 28,29,31
X	EP 0 869 187 A (BECTON DICKINSON CO) 7 October 1998 (1998-10-07) the whole document ---	22-25, 27-29,31
X	EP 0 524 808 A (HOFFMANN LA ROCHE ;UNIV NEW YORK (US)) 27 January 1993 (1993-01-27) abstract page 6, line 27 - line 43 ---	22-25, 27-29,31
X	RIGLER MARY N ET AL: "Differences in the mechanism of stimulation of T7 DNA polymerase by two binding modes of Escherichia coli single-stranded DNA-binding protein" JOURNAL OF BIOLOGICAL CHEMISTRY,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD,US, vol. 270, no. 15, 1995, pages 8910-8919, XP002155011 ISSN: 0021-9258 the whole document ---	22-29,31
X	SCHWARZ K ET AL: "IMPROVED YIELDS OF LONG PCR PRODUCTS USING GENE 32 PROTEIN" NUCLEIC ACIDS RESEARCH,OXFORD UNIVERSITY PRESS, SURREY,GB, vol. 18, no. 4, 1990, page 1079 XP000971373 ISSN: 0305-1048 the whole document ---	22-29,31
X	OSHIMA R O: "SINGLE-STRANDED DNA BINDING PROTEIN FACILITATES AMPLICATION OF GENOMIC SEQUENCES BY PCR" BIOTECHNIQUES,EATON PUBLISHING, NATICK,US, vol. 13, no. 128, 1992, page 188 XP000971377 ISSN: 0736-6205 the whole document -----	22-29,31

INTERNATIONAL SEARCH REPORT

International application No.
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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

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.:

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: 1-21, 30

A method to increase the processivity of RNA- or DNA dependent polymerases comprising adding a general RNA binding protein to a nucleic acid polymerization mixture; an improved method of cDNA synthesis comprising adding a general RNA binding protein to a nucleic acid polymerization mixture and methods to identify agents which can increase the processivity of RNA- or DNA-dependent polymerases.

2. Claims: 22-29, 31

A method to increase the processivity of DNA-dependent RNA or DNA polymerases comprising adding a general DNA binding protein to a nucleic acid polymerization mixture; an improved method of cDNA synthesis comprising adding a general DNA binding protein to a nucleic acid polymerization mixture and a method to increase the processivity of RNA-dependent DNA polymerases comprising adding a general DNA binding protein to a nucleic acid polymerization mixture.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 00/00261

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 4331589	A	25-05-1982	US 4224408 A	23-09-1980
EP 0821059	A	28-01-1998	JP 10033174 A	10-02-1998
			CA 2211367 A	25-01-1998
			JP 10084961 A	07-04-1998
			US 6013488 A	11-01-2000
WO 9117267	A	14-11-1991	US 5223414 A	29-06-1993
			CA 2056983 A	08-11-1991
			DE 69122712 D	21-11-1996
			DE 69122712 T	20-02-1997
			EP 0481065 A	22-04-1992
			WO 9305178 A	18-03-1993
			JP 3010738 B	21-02-2000
			JP 4507198 T	17-12-1992
			US 5670316 A	23-09-1997
			US 5273881 A	28-12-1993
EP 0869187	A	07-10-1998	CA 2224120 A	24-08-1998
			JP 10234389 A	08-09-1998
EP 0524808	A	27-01-1993	AU 645915 B	27-01-1994
			AU 2041992 A	25-02-1993
			CA 2074214 A	24-01-1993
			CA 2218875 A	24-01-1993
			DE 69227076 D	29-10-1998
			DE 69227076 T	02-06-1999
			DK 524808 T	14-06-1999
			EP 0863213 A	09-09-1998
			ES 2123542 T	16-01-1999
			JP 2084412 C	23-08-1996
			JP 5336971 A	21-12-1993
			JP 8000073 B	10-01-1996
			US 5538871 A	23-07-1996