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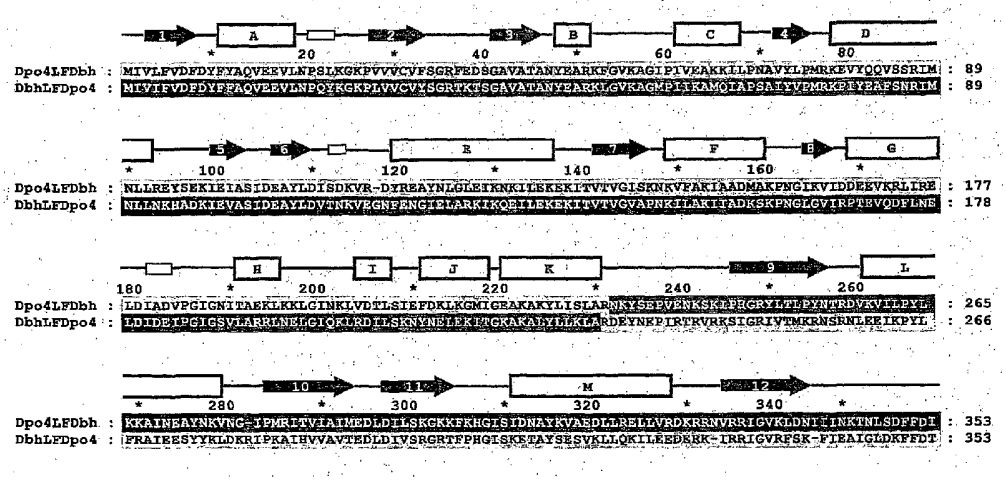
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[Continued on next page]

(54) Title: THERMOSTABLE Y-FAMILY POLYMERASES AND CHIMERAS



(57) **Abstract:** The present disclosure is related to thermostable Y-family polymerases, in particular several novel Y-family polymerases and chimeras made therefrom, as well as methods of identifying other Y-family polymerases, methods of generating other chimeric Y-family polymerases, methods of amplifying ancient or damaged DNA, and methods of incorporating fluorescent or modified nucleotides into a DNA molecule.

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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.

INTERNATIONAL SEARCH REPORT

US2005/017941

A. CLASSIFICATION OF SUBJECT MATTER
C12N9/12 C12N15/54 C12N15/62 C12N5/10 C12P19/34

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)
C12N C12P

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, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LING HONG ET AL: "Crystal structure of a Y-family DNA polymerase in action: A mechanism for error-prone and lesion-bypass replication" CELL, vol. 107, no. 1, 5 October 2001 (2001-10-05), pages 91-102, XP002342865 ISSN: 0092-8674 cited in the application the whole document -/--	1-22, 24-27

[X] 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

"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

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"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
15 November 2005	01. 12. 2005

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	Kools, P

INTERNATIONAL SEARCH REPORT

PCT/US2005/017941

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OHMORI HARUO ET AL: "The Y-family of DNA polymerases" MOLECULAR CELL, vol. 8, no. 1, July 2001 (2001-07), pages 7-8, XP002342866 ISSN: 1097-2765 cited in the application the whole document	1-22, 24-27
X	YANG WEI: "Damage repair DNA polymerases Y." CURRENT OPINION IN STRUCTURAL BIOLOGY, vol. 13, no. 1, February 2003 (2003-02), pages 23-30, XP002354131 ISSN: 0959-440X the whole document	1-22, 24-27
X	KULAEVA OLGA I ET AL: "Identification of a DinB/UmuC homolog in the archaeon Sulfolobus solfataricus" MUTATION RESEARCH, vol. 357, no. 1-2, 1996, pages 245-253, XP002342867 ISSN: 0027-5107 cited in the application abstract	1-22, 24-27
A	LING HONG ET AL: "Snapshots of replication through an abasic lesion: Structural basis for base substitutions and frameshifts." MOLECULAR CELL, vol. 13, no. 5, 12 March 2004 (2004-03-12), pages 751-762, XP002342868 ISSN: 1097-2765 the whole document	1-22, 24-27
A	JOHNSON ROBERT E ET AL: "Deoxynucleotide triphosphate binding mode conserved in Y family DNA polymerases." MOLECULAR AND CELLULAR BIOLOGY, vol. 23, no. 8, April 2003 (2003-04), pages 3008-3012, XP002342869 ISSN: 0270-7306 the whole document	1-22, 24-27

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INTERNATIONAL SEARCH REPORT

PCT/US2005/017941

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KOKOSKA ROBERT J ET AL: "Low fidelity DNA synthesis by a Y family DNA polymerase due to misalignment in the active site" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 22, 31 May 2002 (2002-05-31), pages 19633-19638, XP002342870 ISSN: 0021-9258 the whole document	1-22, 24-27
P,X	BOUDSOCQ FRANCOIS ET AL: "Investigating the role of the little finger domain of Y-family DNA polymerases in low fidelity synthesis and translesion replication" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 279, no. 31, 30 July 2004 (2004-07-30), pages 32932-32940, XP002342871 ISSN: 0021-9258 the whole document	1-22, 24-27

INTERNATIONAL SEARCH REPORT

PCT/US2005/017941

Box 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 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:

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.:

1-22, 24-27

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: 1-22, 24-27 (all partial)

   Isolated Y-family polymerase with Seq ID No 6 or sequences having at least 95% identity and/or conservative variants thereof. Polynucleotides encoding said polypeptides and polynucleotide with Seq ID No 5. Chimeric proteins comprising part of Seq ID No 6 (Seq ID 57, 59), or sequences having 95% identity or conservative variants thereof. Polynucleotide with Seq ID No 56 or 58 or variants thereof. Recombinant polynucleotides comprising said sequences operably linked to a promoter sequence. Cells transformed with said recombinant nucleic acid. Method of generating chimeric Y-family polymerases. Method of using said polymerase in a nucleotide addition reaction. Method of amplifying a DNA molecule using said polymerase.

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2. claims: 1-9, 19-22, 24-27 (all partial)

   As for subject 1, now for Seq ID No 8 and encoding polynucleotide with Seq ID No 7.

   ---

3. claims: 1-9, 19-22, 24-27 (all partial)

   As for subject 1, now for Seq ID No 10 and encoding polynucleotide with Seq ID No 9.

   ---

4. claims: 1-9, 19-22, 24-27 (all partial)

   As for subject 1, now for Seq ID No 12 and encoding polynucleotide with Seq ID No 11.

   ---

5. claims: 1-22, 24-27 (all partial)

   As for subject 1, now for Seq ID No 14 and encoding polynucleotide with Seq ID No 13.

   ---

6. claims: 1-9, 19, 24-27 (all partial)

   As for subject 1, now for Seq ID No 41 and encoding polynucleotide with Seq ID No 40.

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7. claims: 1-9, 19, 24-27 (all partial)

   As for subject 1, now for Seq ID No 43 and encoding polynucleotide with Seq ID No 42.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. claims: 10-22 (all partial)

Isolated chimeric Y-family polymerase with Seq ID No 2 or 4 or sequences having at least 95% identity and/or conservative variants thereof. Polynucleotides encoding said polypeptides and polynucleotide with Seq ID No 1 or 3. Recombinant polynucleotides comprising said sequences operably linked to a promoter sequence. Cells transformed with said recombinant nucleic acid. Method of generating chimeric Y-family polymerases. Method of using the chimeric polymerase in a polymerase reaction. Method of generating chimeric Y-family polymerases wherein the first polymerase is Dbh from Sulfolobus acidocaldarius and the second is from Dpo4 from Sulfolobus solfactaricus or the other way around. Method of using the chimeric polymerase for the addition of a nucleotide.

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9. claim: 23

Method of identifying a Y-family polymerase using degenerate primers.
