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- (71) Applicant: THE UNIVERSITY OF SHEFFIELD [GB/GB]; University of Sheffield, Western Bank, Firth Court, Sheffield, Yorkshire S10 2TN (GB).
- (72) Inventors: SAYERS, Jon; University of Sheffield, Western Bank, Firth Court, Sheffield, Yorkshire S10 2TN (GB). ZHANG, Jing; University of Oxford, MRC Molecular Haematology Unit, The Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford Oxfordshire OX3 9D2 (GB).
- (74) Agent: SYMBIOSIS IP LIMITED; Dr Astrid Lorenz, Apollo House, Eboracum Way, Heworth Green, York Yorkshire YO31 7RE (GB).
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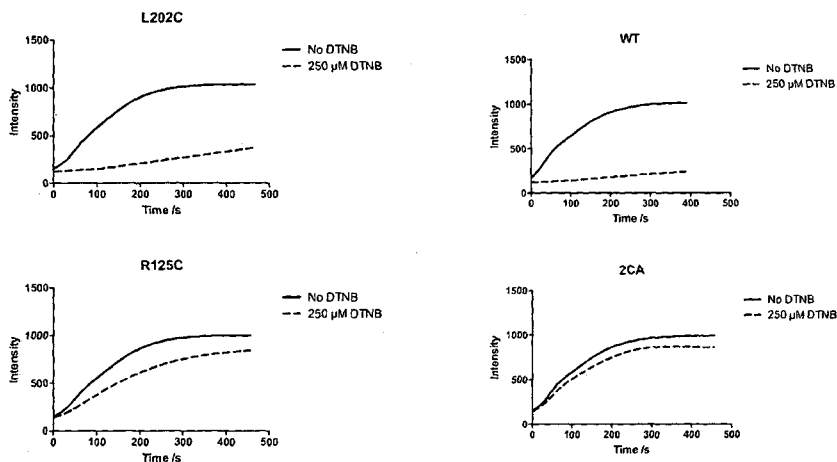
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) Title: MODIFIED EXONUCLEASE



(57) Abstract: The disclosure relates to modified exonuclease enzymes and their use in DNA manipulation.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/052922

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/22 C12N11/12
ADD.
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
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)
EPO-Internal, CHEM ABS Data, Sequence Search, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. J. GARFORTH ET AL: "Structure-Specific DNA Binding by Bacteriophage T5 5'→3' Exonuclease", NUCLEIC ACIDS RESEARCH, vol. 25, no. 19, 1 October 1997 (1997-10-01), pages 3801-3807, XP55056320, ISSN: 0305-1048, DOI: 10.1093/nar/25.19.3801 cited in the application	1,2, 22-41
Y	page 3804, column 1, paragraph 1	6-8, 11-13
A	----- -/--	3-5,9, 10,14, 15,42-47

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 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

"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 3 June 2013	Date of mailing of the international search report 11/06/2013
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Lonnoy, Olivier

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2012/052922

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2007/041361 A1 (US GOV HEALTH & HUMAN SERV [US]; NEWTON DIANNE L [US]; NELLIS DAVID F) 12 April 2007 (2007-04-12) claims 1,12,25	6-8, 11-13
A	----- MIN FENG ET AL: "Roles of divalent metal ions in flap endonuclease-substrate interactions", NATURE STRUCTURAL & MOLECULAR BIOLOGY, vol. 11, no. 5, 1 May 2004 (2004-05-01), pages 450-456, XP55056205, ISSN: 1545-9993, DOI: 10.1038/nsmb754 cited in the application figures 3,4; table 2	1
X	----- US 2003/087278 A1 (SAYERS JON [GB] ET AL) 8 May 2003 (2003-05-08) claim 1; figure 2	43
X	----- WO 96/41005 A1 (MURTAGH JAMES J [US]; THUNNISSEN FREDERIK B J M [NL]) 19 December 1996 (1996-12-19) page 56, line 5 - line 10	43,44
X	----- WO 00/17320 A2 (AMBION INC [US]; PASLOSKE BRITTAN L [US]; WU WILLIAM [US]) 30 March 2000 (2000-03-30) claim 4	43,44
X	----- WO 2006/138558 A2 (WISCONSIN ALUMNI RES FOUND [US]; RAINES RONALD T [US]; PHILLIPS GEORGE) 28 December 2006 (2006-12-28) paragraph [0086]	43-45
X	----- C.-Y. CHOW ET AL: "Compact dimension of denatured states of staphylococcal nuclease", PROTEINS: STRUCTURE, FUNCTION, AND BIOINFORMATICS, vol. 72, no. 3, 15 August 2008 (2008-08-15), pages 901-909, XP55065075, ISSN: 0887-3585, DOI: 10.1002/prot.21985 abstract	43-45
A	----- WO 2010/086603 A1 (OXFORD NANOPORE TECHNOLOGIES L [GB]; MOYSEY RUTH [GB]; KNAGGS MICHAEL) 5 August 2010 (2010-08-05) claims 11,12	46,47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2012/052922

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:

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 fees, this Authority did not invite payment of 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.:

1-15, 43-47(completely); 22-42(partially)

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 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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-15, 46, 47(completely); 22-45(partially)

Exonuclease including a substitution of cysteine 115 of SeqIdNo.1 with an amino acid selected from alanine, glycine, threonine or valine and/or a substitution of cysteine 266 of SeqIdNo.1 with an amino acid selected from alanine or glycine; said exonuclease coupled to a solid phase support matrix; nucleic acid encoding said exonuclease; vector, cell, culture vessel and recombinant method relating thereto.

2. claims: 16-21(completely); 22-42(partially)

Exonuclease comprising a modified amino acid sequence, including modification of Aspartic Acid 155 of SeqIdNo.1; said exonuclease coupled to a solid phase support matrix; nucleic acid encoding said exonuclease; vector, cell, culture vessel and recombinant method relating thereto.

3. claims: 43-45(partially)

Method for the modification of an exonuclease comprising contacting said exonuclease with an agent that reacts with one or more thiol-containing amino acids in said exonuclease.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2012/052922

Patent document cited in search report	Publication date	Publication date	Patent family member(s)	Publication date
WO 2007041361	A1	12-04-2007	NONE	

US 2003087278	A1	08-05-2003	NONE	

WO 9641005	A1	19-12-1996	AU 6256896 A	30-12-1996
			US 5744306 A	28-04-1998
			WO 9641005 A1	19-12-1996

WO 0017320	A2	30-03-2000	AU 6162599 A	10-04-2000
			CA 2360539 A1	30-03-2000
			EP 1151132 A2	07-11-2001
			US 6777210 B1	17-08-2004
			US 2002026046 A1	28-02-2002
			WO 0017320 A2	30-03-2000

WO 2006138558	A2	28-12-2006	EP 1896579 A2	12-03-2008
			JP 2008546391 A	25-12-2008
			US 2006292137 A1	28-12-2006
			US 2009311784 A1	17-12-2009
			US 2012009173 A1	12-01-2012
			US 2013011904 A1	10-01-2013
			WO 2006138558 A2	28-12-2006

WO 2010086603	A1	05-08-2010	AU 2010209508 A1	18-08-2011
			CA 2750874 A1	05-08-2010
			CN 102439043 A	02-05-2012
			CN 102482330 A	30-05-2012
			EP 2391639 A1	07-12-2011
			EP 2391655 A1	07-12-2011
			JP 2012516145 A	19-07-2012
			KR 20110125226 A	18-11-2011
			US 2012064599 A1	15-03-2012
			US 2012100530 A1	26-04-2012
			WO 2010086602 A1	05-08-2010
			WO 2010086603 A1	05-08-2010
