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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.

(54) Title: PH TOLERANT LUCIFERASE

(57) Abstract: Use of a luciferase that has a mutation of at least one amino acid selected from the group consisting of positions 14, 35, 182, 232 and 465, where the numbering is according to the sequence of the luciferase from *P. pyralis* (SEQ ID NO:1) in a method that is performed at a pH below the optimal pH for the wild-type luciferase during at least part of the time period over which bioluminescence measurements are taken, wherein the specific activity of the mutant luciferase is higher than the specific activity of wild-type at the pH at which the method is carried out.



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INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/66 C12N9/02

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)
C12Q 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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/232404 A1 (WOOD KEITH V [US] ET AL) 18 December 2003 (2003-12-18) cited in the application	2-16, 18-25
Y	figures 58,54; table 1 -----	27-29
Y	WO 2004/062338 A2 (TISI LAURENCE C [GB]; MURRAY JAMES A H [GB]) 29 July 2004 (2004-07-29) cited in the application the whole document ----- -/-	27-29

☒ 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 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

- "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

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INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2006/002974

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TISI L C ET AL: "Development of a thermostable firefly luciferase" ANALYTICA CHIMICA ACTA, vol. 457, no. 1, 15 April 2002 (2002-04-15), pages 115-123, XP002424745 ISSN: 0003-2670 cited in the application table 2	1, 18
A	WO 00/24878 A (SECR DEFENCE [GB]; SQUIRRELL DAVID JAMES [GB]; MURPHY MELENIE JANE [GB] 4 May 2000 (2000-05-04)	
A	KITAYAMA ATSUSHI ET AL: "Creation of a thermostable firefly luciferase with pH-insensitive luminescent color." PHOTOCHEMISTRY AND PHOTOBIOLOGY, vol. 77, no. 3, March 2003 (2003-03), pages 333-338, XP009080342 ISSN: 0031-8655	
P,X	LAW G H ERICA ET AL: "Mutagenesis of solvent-exposed amino acids in Photinus pyralis luciferase improves thermostability and pH-tolerance" BIOCHEMICAL JOURNAL, vol. 397, no. Part 2, July 2006 (2006-07), pages 305-312, XP002424746 ISSN: 0264-6021 the whole document	1-17, 20-24
A	WO 2004/059294 A (PROMEGA CORP [US]; HAWKINS ERIKA [US]; CALI JAMES J [US]; HO SAMUEL KI) 15 July 2004 (2004-07-15) cited in the application	
A	HIROKAWA K ET AL: "Improved practical usefulness of firefly luciferase by gene chimerization and random mutagenesis" BIOCHIMICA ET BIOPHYSICA ACTA. PROTEIN STRUCTURE AND MOLECULAR ENZYMOLOGY, ELSEVIER, AMSTERDAM,, NL, vol. 1597, no. 2, 3 June 2002 (2002-06-03), pages 271-279, XP004361031 ISSN: 0167-4838	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2006/002974

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.:
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 and 17 (completely), 2-16, 20-24 (partially)

Subject-matter relating to the use of a luciferase that has a mutation at at least one of the positions 14, 35, 182, 232, and 465, where the numbering is according to the sequence of the luciferase of the firefly *Photinus pyralis* (SEQ. ID. No. 1) in a method that is performed at a pH below the optimal pH for the wild-type luciferase during at least part of the time period over which bioluminescence measurements are taken, wherein the specific activity of the mutant luciferase is higher than the specific activity of wild-type at the pH at which the method is carried out.

2. claims: 18, 19, 25-29 (completely), 2-16, 20-24 (partially)

Subject-matter relating to a luciferase showing a large pH and temperature tolerance but which has not a specific activity higher than the wild-type, wherein the luciferase has a mutation at at least one of the positions 14, 35, 182, 232, and 465, where the numbering is according to the sequence of the luciferase of the firefly *Photinus pyralis* (SEQ. ID. No. 1) and one mutation at least one of the positions 105, 214, 215, 295, 234, 354, 357 and 420 and its use in bioluminescence assays.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2006/002974

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 2003232404	A1	18-12-2003	NONE	
WO 2004062338	A2	29-07-2004	AU 2004204396 A1	29-07-2004
			CA 2513073 A1	29-07-2004
			CN 1852990 A	25-10-2006
			EP 1585836 A2	19-10-2005
			JP 2006516891 T	13-07-2006
			US 2006257874 A1	16-11-2006
WO 0024878	A	04-05-2000	AU 770722 B2	26-02-2004
			AU 6357399 A	15-05-2000
			AU 2004202276 A1	17-06-2004
			AU 2004202277 A1	17-06-2004
			CA 2342300 A1	04-05-2000
			EP 1124944 A2	22-08-2001
			GB 2345913 A	26-07-2000
			JP 2003518912 T	17-06-2003
WO 2004059294	A	15-07-2004	AU 2003300008 A1	22-07-2004
			CA 2508072 A1	15-07-2004
			EP 1588143 A2	26-10-2005
			JP 2006517401 T	27-07-2006