

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 September 2007 (07.09.2007)

PCT

(10) International Publication Number
WO 2007/100392 A3

(51) International Patent Classification:
C12Q 1/00 (2006.01)

(21) International Application Number:
PCT/US2006/061167

(22) International Filing Date:
21 November 2006 (21.11.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/741,263 30 November 2005 (30.11.2005) US

(71) Applicant (for all designated States except US): **BIOTIUM, INC.** [US/US]; 3423 Investment Boulevard, Suite 8, Hayward, California 94545 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MAO, Fei** [US/US]; 34379 Benedick Lane, Fremont, California 94555 (US). **CEN, Hui** [US/US]; 5142 Masonic Avenue, Oakland, California 94618 (US). **LEUNG, Wai-ye** [US/US]; 3083 Sandy Way, San Ramon, California 94583 (US).

(74) Agent: **DE RUNTZ, Alison, K.**; Davis Wright Tremaine LLP, 505 Montgomery Street, Suite 800, San Francisco, California 94111 (US).

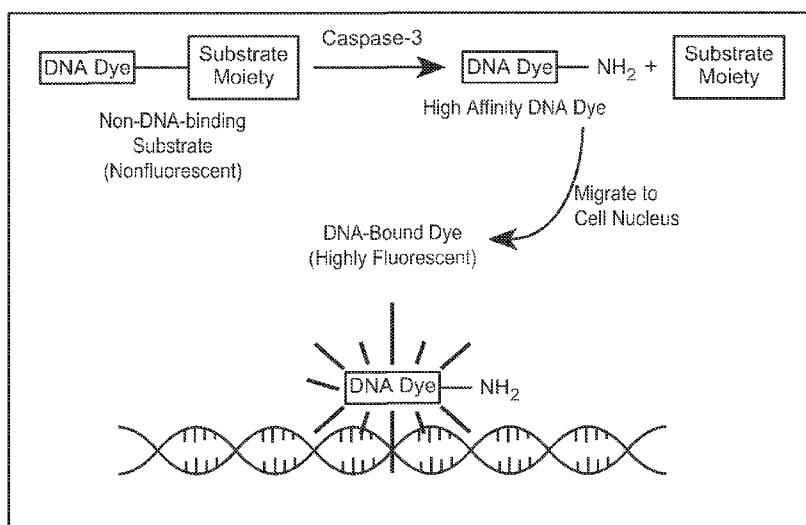
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

[Continued on next page]

(54) Title: ENZYME SUBSTRATE COMPRISING A FUNCTIONAL DYE AND ASSOCIATED TECHNOLOGY AND METHODS



(57) Abstract: Enzyme substrates and associated technology of the present invention are provided. An enzyme substrate of the invention may comprise a biologically functional fluorescent dye and an enzyme-specific substrate moiety attached in such a way that the functionality of the functional dye is diminished. An enzymatic reaction may cleave at least a portion of the substrate moiety from the enzyme substrate to provide a more functional product dye. This product dye may be nonfluorescent or weakly fluorescent, in general, and relatively fluorescent, in a particular condition, such as when bound to a partner biological molecule or an assembly of partner biological molecules. An enzyme substrate of the present invention may thus be useful in fluorescence detection, and/or in any of a variety of useful applications, such as the detection of enzymatic activity in a cell-free system or in a living cell, the screening of drugs, or the diagnosis of disease.

WO 2007/100392 A3



— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:

24 July 2008

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US06/61167

A. CLASSIFICATION OF SUBJECT MATTER IPC: C12Q 1/00(2006.01) USPC: 435/4 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) U.S. : 435/4 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)					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.			
A	US 6,956,032 B1 (WAGGONER) 18 October 2005 (18.10.2005).	1-72			
A	US 2005/0272053 A1 (MAO et al) 8 December 2005 (08.12.2005).	1-72			
A	US 5,321,130 A (YUE et al) 14 June 1994 (14.06.1994).	1-72			
A	US 5,582,977 A (YUE et al) 10 December 1996 (10.12.1996).	1-72			
A	US 5,741,657 A (TSIEN et al) 21 April 1998 (21.04.1998).	1-72			
A	US 5,955,604 A (TSIEN et al) 21 September 1999 (21.09.1999).	1-72			
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.					
<table border="0"> <tr> <td colspan="2"> * 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 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 </td> <td> "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 </td> </tr> </table>			* 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 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
* 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 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 04 June 2008 (04.06.2008)		Date of mailing of the international search report 13 JUN 2008			
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201		Authorized officer AMANDA P. WOOD <i>J. Roberts for</i> Telephone No. 703-308-0196			