(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 June 2004 (03.06.2004)

PCT

(10) International Publication Number
WO 2004/046730 A3

(51) International Patent Classification[7]: G01N 33/94, A61K 47/48, C07G 11/00, C07K 14/00, G01N 33/58, 33/533

(21) International Application Number: PCT/IB2003/005430

(22) International Filing Date: 27 October 2003 (27.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0224872.2	25 October 2002 (25.10.2002)	GB
0229640.8	20 December 2002 (20.12.2002)	GB
0307379.8	31 March 2003 (31.03.2003)	GB

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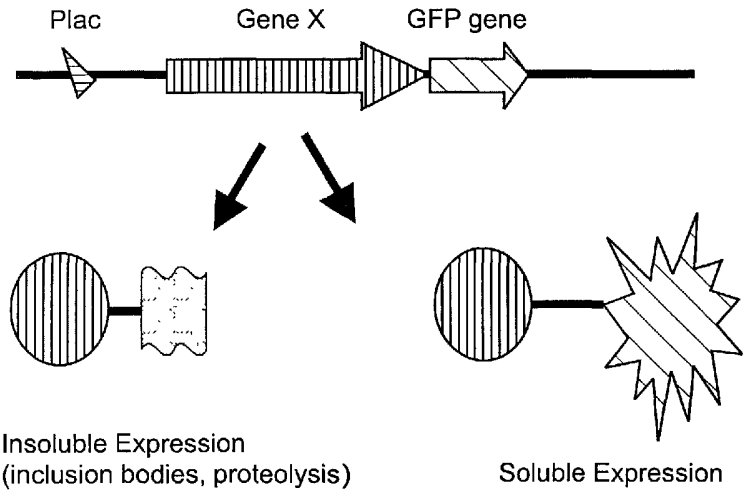
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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,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

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: USES OF BLE PROTEINS AND ANTIBIOTICS FROM THE BLEOMYCIN FAMILY



(57) Abstract: The present invention provides use of a protein conjugate comprising a "ble" protein, which has specific binding properties. The protein conjugates are capable of binding reversibly to an antibiotic from the bleomycin family, which property is exploited in a variety of immobilisation methods. In preferred aspects of the invention, the conjugates are used as markers for protein expression and/or folding, or for affinity tagging. The present invention also provides a probe comprising an array of an immobilised antibody from the bleomycin family, which acts as an analyte capture moiety. In another aspect, a purification media is provided, which comprises an antibiotic of the bleomycin family as an analyte capture moiety. Also provided is a method for generating soluble forms of an insoluble protein by expressing the protein as a "ble" fusion protein and selecting in the presence of an antibiotic from the bleomycin family. In a further aspect, the "ble" protein is expressed as a fusion protein in a cell into which is introduced a labelled antibiotic of the bleomycin family, thereby allowing identification of the cellular localisation of the protein.

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Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

(88) Date of publication of the international search report:
2 September 2004

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

ernational Application No
PCT/IB 03/05430

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/94 A61K47/48 C07G11/00 C07K14/00 G01N33/58
G01N33/533

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 G01N

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, PAJ, 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	BENNETT R P ET AL: "Fusion of green fluorescent protein with the Zeocin-resistance marker allows visual screening and drug selection of transfected eukaryotic cells" BIOTECHNIQUES, EATON PUBLISHING, NATICK, US, vol. 24, no. 3, March 1998 (1998-03), pages 478-482, XP002114401 ISSN: 0736-6205 figure 1 the whole document ----- -/--	1,2,5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 May 2004	03/06/2004

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Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

national Application No
PCT/IB 03/05430

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 02/27327 A (SENSE PROTEOMIC LTD ; SAMADDAR MITALI (GB); BLACKBURN JONATHAN MICHAEL) 4 April 2002 (2002-04-04) the whole document pages 15-17 claim 9	3,4
X	US 5 420 228 A (MISTRY JEHANGIR S ET AL) 30 May 1995 (1995-05-30) the whole document claims 5,6	29-32
X	CALMELS THIERRY P G ET AL: "Nuclear localization of bacterial Streptoalloteichus hindustanus bleomycin resistance protein in mammalian cells" MOLECULAR PHARMACOLOGY, vol. 44, no. 6, 1993, pages 1135-1141, XP008030691 ISSN: 0026-895X the whole document page 1139, column 1, paragraph 2	29-32
A	US 6 027 900 A (GROSSMAN ARTHUR R ET AL) 22 February 2000 (2000-02-22) the whole document	33-35

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INTERNATIONAL SEARCH REPORT

rnational Application No
PCT/IB 03/05430

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0227327 A	04-04-2002	AU 7994801 A	08-04-2002
		BR 0113330 A	30-12-2003
		CA 2420415 A1	04-04-2002
		EP 1326970 A2	16-07-2003
		WO 0227327 A2	04-04-2002
		GB 2384779 A	06-08-2003
		US 2003073811 A1	17-04-2003
US 5420228 A	30-05-1995	NONE	
US 6027900 A	22-02-2000	WO 9739106 A1	23-10-1997