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- (71) **Applicant (for all designated States except US):** BIO-GLOW INC. [US/US]; 175 Harbor Road, Head Of The Harbor, NY 11780 (US).
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- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,

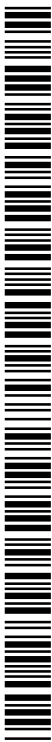
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(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, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(88) **Date of publication of the international search report:**  
10 May 2013



WO 2011/106001 A3

(54) **Title:** AUTOLUMINESCENT PLANTS INCLUDING THE BACTERIAL LUX OPERON AND METHODS OF MAKING SAME

**Figure 12**

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Truncated -----CCGCGTCTTCAATGAGAAATGCAATAGAGGCTCTCTGG 88
Shimizu et al. GCGTACGTGAGGTTCTGCTCCCGCCCGCTCTGCAATGAGAAATGCAATAGAGGCTCTCTGG 68
Lutz et al. -----TATGATCCCGCTCCCGCCCGCTCTGCAATGAGAAATGCAATAGAGGCTCTCTGG 54
*****

Truncated AATGACGTGAGGGGGCAGGGATGCTATATTTCTGGGAGCGAATC ---CCGGGGAAAT 95
Shimizu et al. AATGACGTGAGGGGGCAGGGATGCTATATTTCTGGGAGCGAATC ---CCGGGGAAAT 117
Lutz et al. AATGACGTGAGGGGGCAGGGATGCTATATTTCTGGGAGCGAATC ---CCGGGGAAAT 114
*****

Truncated CCA-----TCTA---GTCTAGGGAGGATTT 119 (SEQ ID NO: 43)
Shimizu et al. GAAGGCTCTGGATCA---GTCTAGGGAGGATTT 150 (SEQ ID NO: 30)
Lutz et al. GAARATCTATTCATGAGTTGTAGGGAGGATTT 150 (SEQ ID NO: 31)
* * * * *

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(57) **Abstract:** In one aspect, the invention relates to a transgenic autoluminescent plant including an expressible heterologous nucleotide sequence comprising a bacterial LUX operon, which includes LUX A. LUX B. LUX C. LUX D. LUX b. And LUX G genes, wherein the heterologous nucleotide sequence is expressed to render the plant autonomously luminescent.

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 10/25366

<p><b>A. CLASSIFICATION OF SUBJECT MATTER</b>                  IPC(8) - C12N 15/82; A01H 9/00 (2010.01)                  USPC - 800/282; 800/287; 800/295                  According to International Patent Classification (IPC) or to both national classification and IPC</p>																	
<p><b>B. FIELDS SEARCHED</b></p> <p>Minimum documentation searched (classification system followed by classification symbols)                  IPC(8)- C12N 15/82; A01H 9/00 (2010.01)                  USPC- 800/282; 800/287; 800/295</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched                  USPC- 800/288; 800/294; 800/300; 800/303; 435/419; 800/295; 800/303; 800/278; 435/189; 435/468; 435/469; 435/69.8; 435/8</p> <p>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)                  PubWest, DialogPRO (Chemical Engineering and Biotechnology Abstracts, INSPEC, NTIS (National Technical Information Service), PASCAL, Current Contents Search, MEDLINE): Transgenic plant, Prm promoter, heterologous, plastid, luxa luxb luxc luxd luxe luxg                  GenCore 6.3: SEQ ID NO:32, 40, 42, 43</p>																	
<p><b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b></p> <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>Y</td> <td>US 7,663,022 B1 (Hudkins) 16 February 2010 (16.02.2010) col 3, ln 25-27; col 7, ln 44; col 16, ln 45; claim 1</td> <td>1, 2, 4, 6, 8-15</td> </tr> <tr> <td>Y</td> <td>US 7,176,355 B2 (Maliga et al.) 13 February 2007 (13.02.2007) col 2, ln 14-16; col 5, ln 12-13; col 11, ln 23; col 15, ln 38-39; SEQ ID NO 38; Fig. 7</td> <td>1, 2, 4, 6, 8-15</td> </tr> <tr> <td>Y</td> <td>WO 2000/061740 A1 (Yuan, et al.) 19 October 2000 (19.10.2000) pg 4, ln 32</td> <td>11</td> </tr> <tr> <td>A</td> <td>WO 2006/108830 A2 (Dubald, et al.) 19 October 2006 (19.10.2006) SEQ ID NO 6</td> <td>3</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	Y	US 7,663,022 B1 (Hudkins) 16 February 2010 (16.02.2010) col 3, ln 25-27; col 7, ln 44; col 16, ln 45; claim 1	1, 2, 4, 6, 8-15	Y	US 7,176,355 B2 (Maliga et al.) 13 February 2007 (13.02.2007) col 2, ln 14-16; col 5, ln 12-13; col 11, ln 23; col 15, ln 38-39; SEQ ID NO 38; Fig. 7	1, 2, 4, 6, 8-15	Y	WO 2000/061740 A1 (Yuan, et al.) 19 October 2000 (19.10.2000) pg 4, ln 32	11	A	WO 2006/108830 A2 (Dubald, et al.) 19 October 2006 (19.10.2006) SEQ ID NO 6	3
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<p><input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/></p>																	
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</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</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"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</td> </tr> <tr> <td>"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)</td> <td>"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</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&amp;" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"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	"E" earlier application or patent but published on or after the international filing date	"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	"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)	"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	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed						
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"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family																
"P" document published prior to the international filing date but later than the priority date claimed																	
<p>Date of the actual completion of the international search 29.06.2010 (29 June 2010)</p>		<p>Date of mailing of the international search report <b>05 NOV 2010</b></p>															
<p>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</p>		<p>Authorized officer:                  Lee W. Young                  PCT Helpdesk: 571-272-4300                  PCT OSP: 571-272-7774</p>															

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/25366

**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:

Group I+: claims 1-15, drawn to a transgenic plant comprising a vector system comprising a heterologous nucleotide sequence, which comprises LUX A, LUX B, LUX C, LUX D, LUX E, and LUX G genes, wherein the heterologous nucleotide sequence is operably linked to a truncated Prm promoter; and wherein the heterologous nucleotide sequence is capable of being incorporated into a plastid genome. The first invention is restricted to the plant comprising Prm promoter of SEQ ID NO:32, wherein said SEQ ID NO:32 comprises a substitution of the position 3. Should an additional fee(s) be paid, Applicant is invited to elect an additional substitution(s) to be searched. The exact claims searched will depend on the specifically elected substitution(s).

--Please see continuation--

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 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.:
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.: 1-15, restricted to SEQ ID NO: 32 with a substitution in the position 3

**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.

## \*\*\*\*\* SUPPLEMENTAL BOX \*\*\*\*\*

Continuation of Box III

Group II, claims 16-23, drawn to a vector system comprising:

- a) a plastid transformation vector having a first heterologous nucleotide sequence comprising a bacterial LUX operon, which comprises LUX A, LUX B, LUX C, LUX D, LUX E, and LUX G genes, wherein the heterologous nucleotide sequence is operably linked to a first promoter; and wherein the heterologous nucleotide sequence is capable of being incorporated into a plastid genome; and  
b) a vector having a second heterologous nucleotide sequence operably linked to a second promoter.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Group I+ do not include the inventive concept of a vector system comprising a) a plastid transformation vector having a first heterologous nucleotide sequence comprising a bacterial LUX operon, which comprises LUX A, LUX B, LUX C, LUX D, LUX E, and LUX G genes, wherein the heterologous nucleotide sequence is operably linked to a first promoter; and wherein the heterologous nucleotide sequence is capable of being incorporated into a plastid genome; and b) a vector having a second heterologous nucleotide sequence operably linked to a second promoter, as required by Group II.

The inventions of Group II do not include the inventive concept of a truncated Prm promoter specifically defined as SEQ ID NO:32 and a specifically defined as SEQ ID NO:43 heterologous sequence, as required by Group I+.

The inventions of Group I+ share the technical feature of a transgenic plant comprising a vector system comprising a heterologous nucleotide sequence, which comprises LUX A, LUX B, LUX C, LUX D, LUX E, and LUX G genes, wherein the heterologous nucleotide sequence is operably linked to a truncated Prm promoter; and wherein the heterologous nucleotide sequence is capable of being incorporated into a plastid genome. However, this shared technical feature is obvious over prior art as follows:

- 1) US 7,663,022 B1 (Hudkins) (16 February 2010) discloses "[a] transgenic bioluminescent plant, comprising: at least one plant cell comprising at least one recombinant DNA, wherein said at least one plant cell is selected from the group consisting of a monocotyledon cell and a dicotyledon cell; and wherein said at least one recombinant DNA comprises at least one lux gene encoding a luciferase, at least one lux gene encoding a luciferin that is compatible with said luciferase, and at least one light inducible promoter for regulation of expression of said lux genes; and wherein said lux genes are selected from a foreign genome containing a lux operon" (claim 13), and further discloses that said plant comprises "at least one lux gene encoding a luciferase comprises luxA and luxB, and said at least one lux gene encoding a luciferin comprises luxC, luxD and luxE" (claim 15, and further discloses that "[o]ther phototransformative proteins include those encoded by luxF and luxG from the bacterial lux operon" (col 16, ln 54-55).
- 2) US 7,176,355 B2 to Maliga et al., in the context of Plastid rRNA Operon Promoter Elements for Construction of Chimeric Promoters for Transgene Expression (title), a truncated Prm promoter (col 11, ln 30-40) discloses the claimed SEQ ID NO:32 (nucleotides 1-135 of SEQ ID NO 38, please see also Fig 7), and further discloses "FIG. 8 shows block mutagenesis of nucleotides in the wild-type Prm promoter (Wt Prm: nucleotides 18-75 of SEQ ID NO: 3; Prm10: SEQ ID NO: 52) at neutral positions to minimize DNA sequence homology of Prm promoters. The mutant derivative is Prm11 (SEQ ID NO: 51). The sequences shown are suitable for combination with translation control sequences for transgene expression, as described in pending patent application wO 00/07421 for increasing protein expression levels" (col 4, ln 19-27). It would have been obvious to one of ordinary skill in the art to use the truncated Prm promoter disclosed by Maliga et al. for expression of the LUX operon disclosed by Hudkins, because Maliga et al. discloses that said Prm promoter is "useful for driving high level expression of heterologous proteins in plastids" (Abstract). As said transgenic plant was obvious at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The special technical feature of the inventions listed as Group I+ is the specific substitution within SEQ ID NO:32 recited therein. The inventions do not share a special technical feature, because Maliga et al., discloses said SEQ ID NO:32. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.