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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, 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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Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with information concerning request for restoration of the right of priority in respect of one or more priority claims (Rules 26bis.3 and 48.2(b)(vii))
- with sequence listing part of description (Rule 5.2(a))

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

11 April 2013

(54) Title: BACULOVIRAL DNA ELEMENTS FOR THE EXPRESSION OF RECOMBINANT PROTEINS IN A HOST CELL

(57) Abstract: Reagents and methods are provided that allow for an improved expression of a recombinant protein. More specifically, the introduction of recombinant DNA elements into a host cell allows for the increased expression of a recombinant protein, an improvement of the correct folding of said protein and an increase in cell viability and proliferation of the host cell. These recombinant DNA elements can be introduced into host cells, for example, via a recombinant baculovirus, which has incorporated said elements. The recombinant DNA elements include nucleic acids encoding transcriptional regulators, such as IE-0 and IE-1, transcriptional enhancer elements, such as the homologous region (hr) and promoters.



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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☐	on paper
☒	in electronic form
 - b. (time)

☒	in the international application as filed
☐	together with the international application in electronic form
☐	subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/061081

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01K67/033 C12N15/85 C12N15/866
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)
A01K 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, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NAGAI S ET AL: "Comparative transient expression assay analysis of hycu-hr6- and IE1-dependent regulation of baculovirus gp64 early promoters in three insect cell lines", VIRUS RESEARCH, AMSTERDAM, NL, vol. 155, no. 1, 1 January 2011 (2011-01-01), pages 83-90, XP027574274, ISSN: 0168-1702 [retrieved on 2010-12-24] the whole document	1-11
A	US 2009/068703 A1 (CHAO YU-CHAN [TW] ET AL) 12 March 2009 (2009-03-12) the whole document ----- -/-	1



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

25 October 2012

Date of mailing of the international search report

19/02/2013

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/061081

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CROUCH E A ET AL: "Effects of baculovirus transactivators IE-1 and IE-2 on the Drosophila heat shock 70 promoter in two insect cell lines", ARCHIVES OF VIROLOGY ; OFFICIAL JOURNAL OF THE VIROLOGY DIVISION OF THE INTERNATIONAL UNION OF MICROBIOLOGICAL SOCIETIES, SPRINGER-VERLAG, VI, vol. 150, no. 8, 1 August 2005 (2005-08-01), pages 1563-1578, XP019378571, ISSN: 1432-8798, DOI: 10.1007/S00705-005-0527-8 the whole document -----	1-11
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A	W. KANG: "IE1 and hr facilitate the localization of Bombyx mori nucleopolyhedrovirus ORF8 to specific nuclear sites", JOURNAL OF GENERAL VIROLOGY, vol. 86, no. 11, 1 November 2005 (2005-11-01), pages 3031-3038, XP055016117, ISSN: 0022-1317, DOI: 10.1099/vir.0.81270-0 the whole document -----	2
A	LIN X; CHEN Y; YI Y; ZHANG Z: "Baculovirus immediately early 1, a mediator for homologous regions enhancer function in trans", VIROL J, vol. 7, no. 32, 2010, XP002685888, cited in the application the whole document ----- -/--	1-4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/061081

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PASSARELLI ET AL: "Three baculovirus genes involved in late and very late gene expression: ie-1, ie-n, and lef-2.", JOURNAL OF VIROLOGY, vol. 67, no. 4, 1 April 1993 (1993-04-01), pages 2149-2158, XP055016120, ISSN: 0022-538X cited in the application the whole document	1
A	NETTLESHIP J E ET AL: "Recent advances in the production of proteins in insect and mammalian cells for structural biology", JOURNAL OF STRUCTURAL BIOLOGY, ACADEMIC PRESS, UNITED STATES, vol. 172, no. 1, 1 October 2010 (2010-10-01), pages 55-65, XP027249557, ISSN: 1047-8477 [retrieved on 2010-02-11] cited in the application the whole document	1-11
A	S. KANGINAKUDRU ET AL: "Targeting ie-1 gene by RNAi induces baculoviral resistance in lepidopteran cell lines and in transgenic silkworms", INSECT MOLECULAR BIOLOGY, vol. 16, no. 5, 1 October 2007 (2007-10-01), pages 635-644, XP055029022, ISSN: 0962-1075, DOI: 10.1111/j.1365-2583.2007.00753.x the whole document	1-11
A	WO 2010/025764 A1 (ALTERNATIVE GENE EXPRESSION S [ES]; GOMEZ SEBASTIAN SILVIA [ES]; LOPEZ) 11 March 2010 (2010-03-11) the whole document	9
A	V. J. VALDES ET AL: "Using Double-stranded RNA to Prevent in Vitro and in Vivo Viral Infections by Recombinant Baculovirus", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 278, no. 21, 16 May 2003 (2003-05-16), pages 19317-19324, XP055029633, ISSN: 0021-9258, DOI: 10.1074/jbc.M212039200 the whole document	1
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/061081

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OLSON V A ET AL: "The highly conserved basic domain I of baculovirus IE1 is required for hr enhancer DNA binding and hr-dependent transactivation", JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 77, no. 10, 1 May 2003 (2003-05-01), pages 5668-5677, XP002998478, ISSN: 0022-538X cited in the application the whole document	1-4
A	H.-R. LO ET AL: "Novel Baculovirus DNA Elements Strongly Stimulate Activities of Exogenous and Endogenous Promoters", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 7, 8 February 2002 (2002-02-08), pages 5256-5264, XP055011180, ISSN: 0021-9258, DOI: 10.1074/jbc.M108895200 the whole document	1,2
A	CRISTIANO A FELIPE ALVES ET AL: "hycu-hr6, A large homologous region of the Hyphantria cunea nucleopolyhedrovirus genome, as a powerful and versatile enhancer in insect expression systems", VIRUS GENES, KLUWER ACADEMIC PUBLISHERS, BO, vol. 39, no. 3, 7 October 2009 (2009-10-07), pages 403-408, XP019754329, ISSN: 1572-994X, DOI: 10.1007/S11262-009-0406-6 the whole document	2
A	US 5 965 393 A (HASNAIN SEYED E [IN] ET AL) 12 October 1999 (1999-10-12) the whole document	2-6
T	GOMEZ-CASADO E; GOMEZ-SEBASTIAN S; NÚÑEZ MC; LASA-COVARRUBIAS R; MARTÍNEZ-PULGARÍN S; ESCRIBANO JM: "Insect larvae biofactories as a platform for influenza vaccine production", PROTEIN EXPR PURIF., vol. 79, 2011, pages 35-43, XP002685889, the whole document	

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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.:
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.:

1-40

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: 1-40

Recombinant baculovirus comprising a nucleic acid sequence that allows for the expression above endogenous levels of the proteins IE-1, IE-0 and/or fragments thereof functioning as transcriptional regulators, wherein the nucleic acid is selected from the group consisting of: (a) a nucleic acid containing the nucleotide sequence indicated in any of SEQ ID NO: 1-5; b) a nucleic acid sequence having a sequence identity of at least 70%, preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90% and most preferably at least 95% with the nucleotide sequence indicated in any of SEQ ID NO: 1-5 and encoding protein to function as a transcriptional regulator in a recombinant baculovirus; c) a nucleic acid sequence encoding an amino acid containing the amino acid sequence indicated in any of SEQ ID NO: 6-9; and d) a nucleic acid sequence encoding an amino acid sequence having a sequence similarity of at least 70%/75%/80%/ 85%/90%/95% with the amino acid sequence indicated in any of SEQ ID NO: 6-9 and able to function as a transcriptional regulator in a recombinant baculovirus; transfer vector suitable for producing such a recombinant baculovirus, comprising said sequence, further comprising a nucleic acid sequence suitable for integration or transposition in a baculovirus; use of said transfer vector for producing such a recombinant baculovirus; cloning vector suitable for producing such a recombinant baculovirus or transfer vector, comprising said sequence for expression above endogenous levels of the proteins IE-0, IE-1 and/or fragments thereof functioning as transcriptional regulators, which is further suitable for bacterial replication; use of said cloning vector for producing such a recombinant baculovirus or transfer vector; nucleic acid sequence suitable for producing said recombinant baculovirus, transfer vector or cloning vector, comprising said sequence; use thereof for producing such a recombinant baculovirus, transfer vector; or cloning vector; host cell infected, transfected, transduced or transformed with said recombinant baculovirus, transfer vector, cloning vector or nucleic acid sequence; culture medium comprising said recombinant baculovirus, transfer vector, cloning vector or nucleic acid sequence; method for producing a recombinant protein that comprises said infected, transfected, transduced or transformed host cell;

2. claims: 41-43

Transgenic cell line comprising a transgene, which is a nucleic acid selected from the group consisting of:
(a) a nucleic acid containing the nucleotide sequence indicated in any of SEQ ID NO: 1-5;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

(b) a nucleic acid sequence having a sequence identity of at least 70%, preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90% and most preferably at least 95% with the nucleotide sequence indicated in any of SEQ ID NO: 1-5 and encoding a protein able to function as a transcriptional regulator in a recombinant baculovirus;

[c) a nucleic acid sequence encoding an amino acid containing the amino acid sequence indicated in any of SEQ ID NO: 6-9; and

(d) a nucleic acid sequence encoding an amino acid sequence having a sequence similarity of at least 70%, preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90% and most preferably at least 95% with the amino acid sequence indicated in any of SEQ ID NO: 6-9 and able to function as a transcriptional regulator in a recombinant baculovirus.

3. claim: 44

INucleic acid sequence comprising a nucleic acid sequence functioning as a promoter in a recombinant baculovirus, wherein the nucleic acid sequence is selected from the group consisting of:

(a) a nucleic acid containing the nucleotide sequence indicated in SEQ ID NO: 12, 14 or 15; and

(b) a nucleic acid sequence having a sequence identity of at least 70%, preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90% and most preferably at least 95% with the nucleotide sequence indicated in SEQ ID NO: 12, 14 or 15.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/061081

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009068703 A1	12-03-2009	TW 200911993 A US 2009068703 A1	16-03-2009 12-03-2009
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