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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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
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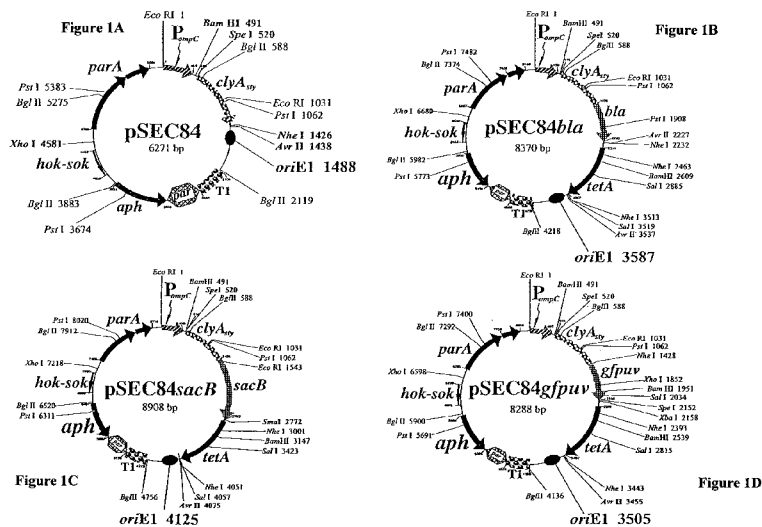
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- (88) **Date of publication of the international search report:**
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(54) **Title:** NON-HEMOLYTIC CLYA FOR EXCRETION OF PROTEINS

Figure 1



(57) **Abstract:** The disclosure below provides a protein export system utilizing non-hemolytic variants of HlyE family member proteins for efficiently producing recombinant protein from a host cell. In a preferred embodiment, the protein export system utilizes protein export machinery endogenous to the host bacterium into which the protein export system vector is introduced.

WO 2009/149083 A3

A. CLASSIFICATION OF SUBJECT MATTER*C07K 19/00(2006.01)i, C12N 15/64(2006.01)i*

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 8 C07K 19/00, C12N 15/64, 5/00, 5/09, A61K 39/00, A61P 31/00, C12P 21/02

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)

eKOMPASS(KIPO internal), PubMed(NCBI), JPO, USPTO, Google

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2002-083890 A2 (James E. Galen) October 24, 2002. See Abstract, Paragraph [0023], [0069], Fig 1-4, Examples 1 and 2.	1, 3 and 9
A	WO 1994-28137 A1 (The United States of America) December 8, 1994. See Abstract, Pages 3-4.	1, 3 and 9
A	Jae-Yong Kim et al. 'Engineered Bacterial Outer Membrane Vesicles with Enhanced Functionality' J. Mol. Biol., Vol. 380, pp.51-66(published online, April 9, 2008). See Abstract, Pages 61-63(Materials and Methods).	1, 3 and 9
T	Christine von Rhein et al. 'ClyA cytolysin from Salmonella: Distribution within the genus, regulation of expression by SlyA, and pore-forming characteristics' International Journal of Medical Microbiology, Vol. 299, pp.21-35(January 2009). See Abstract, Pages 21-22(Introduction).	1, 3 and 9

 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 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

18 JANUARY 2010 (18.01.2010)

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19 JANUARY 2010 (19.01.2010)

Name and mailing address of the ISA/KR

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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.: 2, 4-8 and 10-20
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 2, 4-8 and 10-20 pertain to methods for treatment of the human body by therapy, as well as diagnostic methods, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. Claims Nos.: 8 and 10-18
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:

Though claims 8, 10-18 are dependent on claim 3, claim 3 is different from them in the category. Therefore, claims 8 and 10-18 are unclear.

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:

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 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.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2009/045972

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