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[Continued on next page]

(54) Title: PREPARATION METHOD OF RECOMBINANT PROTEIN BY USE OF A FUSION EXPRESSION PARTNER

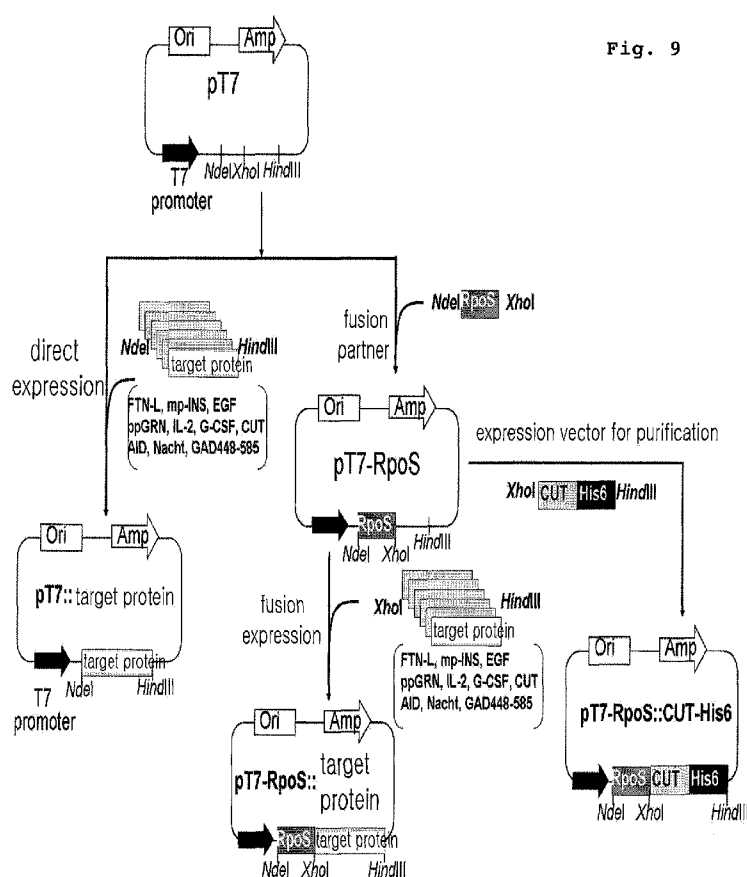


Fig. 9

(57) Abstract: The present invention relates to a preparation method using a fusion expression partner. The method includes preparing a polynucleotide encoding a fusion expression partner selected from the group consisting of SlyD (FKBR- type peptidyl prolyl cis-trans isomerase), Crr (glucose-specific phosphotransferase (PTS) enzyme IIA component), RpoS (RNA polymerase sigma factor), PotD (Spermidine/putrescine-binding periplasmic protein), and RpoA (RNA polymerase alpha subunit), and an expression vector linking a polyDNA fragment of a heterologous protein, preparing a transformant by introducing the expression vector into a host cell, inducing the expression of a recombinant protein by culturing a transformant, and obtaining the expression. In the preparation method of the recombinant protein, the heterologous protein may enhance the water-solubility and folding of the recombinant protein, overcome the limitations about the water-solubility and folding which the conventional fusion expression partners have, and be used widely in the production of pharmaceutical and industrial proteins.



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A. CLASSIFICATION OF SUBJECT MATTER*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: C12N 15/64

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)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Park, J. S. et al. 'Escherichia coli malate dehydrogenase, a novel solubility enhancer for heterologous proteins synthesized I Escherichia coli.' Biotechnology Letters. Vol. 29(10), pp. 1513-1518 (5 June 2007)	1-8
A	Han, K. Y. et al. 'Enhanced solubility of heterologous proteins by fusion expression using stress-induced Escherichia coli protein, Tsf.' FEMS Microbiology Letters. Vol. 274(1), pp. 132-138 (3 July 2007)	1-8
P, X	Han, K. Y. et al. 'Solubilization of aggregation-prone heterologous proteins by covalent fusion of stress-responsive Escherichia coli protein, SlyD.' Protein Engineering, Design, and Selection. Vol. 20(11), pp. 543-549 (30 October 2007) See the Abstract.	1-8
P, X	Park, J. S. et al. 'Solubility enhancement of aggregation-prone heterologous proteins by fusion expression using stress-responsive Escherichia coli protein, RpoS.' BMC Biotechnology. Vol. 8, pp. 15 (19 February 2008) See the Abstract.	1-8
P, X	Han, K. Y. et al. 'Transport proteins PotD and Crr of Escherichia coli, novel fusion partners for heterologous protein expression.' Biochimica et Biophysica Acta. Vol. 1774(12), pp. 1536-1543 (4 October 2007)	1-8



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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2008/005256**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 Extra 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 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.

The separate inventions/groups of inventions are:

Invention 1: a method of producing a recombinant protein using a fusion parter, SlyD. (Claims 1-8 all partially)

Inventions 2-5: As invention 1, but relating to Crr, RpoS, PotD, or RopA.(Claims 1-8 all partially)

The common technical feature among inventions 1-5 is 'a method of producing a recombinant protein using a fusion partner'. Said fusion partner is a protein that response to a stress, and increases solubility when fused to a target protein.

Since those proteins which increase solubility of a target protein are already known from the prior art D1 and D2, the common technical feature is not considered to contribute over the prior art as a whole. Therefore, the present application does not meet the requirements of unity of invention (Rule 13.1 PCT).