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27 November 2008

(54) Title: GENETIC INCORPORATION OF UNNATURAL AMINO ACIDS INTO PROTEINS IN MAMMALIAN CELLS

(57) Abstract: The invention relates to orthogonal pairs of tRNAs and aminoacyl-tRNA synthetases that can incorporate unnatural amino acids into proteins in mammalian host cells, for example, primate host cells and rodent host cells. The invention provides, for example but not limited to, translation systems that include host cells (e.g., primate or rodent cells), orthogonal aminoacyl-tRNA synthetases derived from eubacterial synthetases, orthogonal tRNAs, and the unnatural amino acid. The invention also relates to methods for producing proteins of interest comprising at least one unnatural amino acid in mammalian host cell systems.



WO 2008/073184 A3

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US07/22232

### A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07K 14/00( 2006.01),C07H 21/04( 2006.01)**  
C 12N 15/63( 2006.01)

USPC: 530/350;536/24. 1

According to International Patent Classification (IPC) or to both national classification and IPC

B	FIELDS SEARCHED
1	ALL INFORMATION CONTAINED HEREIN IS UNCLASSIFIED
2	DATE 08-09-2001 BY SP-6 BTJ/KSP
3	REASON FOR DECLASSIFICATION 25X
4	AUTHORITY E.O. 12958
5	COMMENTS
6	DECLASSIFY ON:
7	1 UNLIMITED
8	2 OTHER DATE OR EVENT
9	3 THIS INFORMATION WAS OBTAINED FROM A SOURCE WHOSE
10	IDENTITY AND RELIABILITY ARE NOT KNOWN TO THE
11	DISSEMINATING OFFICE
12	4 DISSEMINATION OF THIS INFORMATION COULD BE DETRIMENTAL TO THE NATIONAL DEFENSE
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Minimum documentation searched (classification system followed by classification symbols)  
U.S. 530/350:536/24. 1

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)  
WEST, STN

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, E	US 2008/0146781 A1 (CHO et al) 19 June 2008 (19 06.2008), paragraphs 239, 308, 603 and 689	1-29

1

Further documents are listed in the continuation of Box C.

**D**

See patent family annex.

*	Special categories of cited documents	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent 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		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search

31 August 2008 (31.08.2008)

Date of mailing of the international search report

16 SEP 2008

Name and mailing address of the ISA/US

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/US07/22232

**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  
Please See Continuation 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 additional fees, this Authority did not invite payment of any 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 1-29 (with the elected species)
- 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

International application No  
PCT/US07/22232

### BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not *so* linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

SEQ ID NO. 57-101 and SEQ ID NO. 8-56. The first group contains SEQ ID NO. 57 and 8. Group 2 would contain SEQ ID NO. 58 and 9, and so forth. SEQ ID NO. 57 and 8 will be examined if no additional fees are paid. Claims 1-29 will be examined with the elected species.

The claims are deemed to correspond to the species listed above in the following manner:

Claims 2, 5, 16, 20 and 9, 22

The following claim(s) are generic: 1 and 15

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons. According to PCT Rule 13.2 and to the guidelines in Section (f)(i)(A) of Annex B of the PCT Administrative Instructions, all alternatives must have a common property or activity and a common structure. The genera of aminoacyl-tRNA synthetases do not have a common structure, because each synthetase contains a unique sequence that results in unique phenotypic properties, resulting in their classification as separate genera in the art. Additionally, each synthetase will have a different activity.