

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
23 September 2010 (23.09.2010)

PCT

(10) International Publication Number
WO 2010/108182 A3

(51) International Patent Classification:
C12P 21/04 (2006.01) *A61K 39/00* (2006.01)

(21) International Application Number:
PCT/US2010/028165

(22) International Filing Date:
22 March 2010 (22.03.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/162,059 20 March 2009 (20.03.2009) US
61/162,030 20 March 2009 (20.03.2009) US
61/255,186 27 October 2009 (27.10.2009) US

(71) Applicant (*for all designated States except US*):
TETRAGENETICS, INC.; One Broadway, 14th Floor,
Cambridge, MA 02142 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **CLARK, Theodore, G.** [US/US]; 206 Fairmount Street, Ithaca, NY 14850 (US). **PAPOYAN, Ashot** [AM/US]; 8 Asbury Road, Lansing, NY 14882 (US). **TURKEWITZ, Aaron** [US/US]; 5410 S. Blackstone Avenue, Chicago, IL 60615 (US).

(74) Agents: **TWOMEY, Michael, J.** et al.; Wilmer Cutler Pickering Hale and Dorr LLP, 60 State Street, Boston, MA 02109 (US).

(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))
- 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 sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:
18 November 2010

(54) Title: PRODUCTION OF RECOMBINANT PROTEINS IN CILIATES AND USES THEREOF

(57) Abstract: This invention is directed to methods for recombinant polypeptide production and, in particular, methods and products for the production and purification of recombinant proteins in ciliates.



WO 2010/108182 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/28165

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12P 21/04; A61K 39/00 (2010.01)

USPC - 435/69.7; 424/191.2

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)

USPC 435/69.7; 424/191.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC 435/69.1 424/184.1 424/192.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST(DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=YES; OP=ADJ), Google Scholar(mucocyst-targeted protein protein production, Gr1 luciferase, Gr1-1 luciferase, Granule lattice protein luciferase, "Granule lattice protein" luciferase, "Granule lattice protein" green fluorescent protein, mucocyst protein production, mucocyst "protein production", mucocyst "h

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	COWAN et al., Genetic, Genomic, and Functional Analysis of the Granule Lattice Proteins in Tetrahymena Secretory Granules. Molecular Biology of the Cell, 2005, vol 16, pp 4046-4060; pg 4048, Expression of GFP-tagged Proteins; pg 4051, fig 5B; pg 4055, fig 9	1-21, 25-26
Y	US 2002/002848 A1 (COUSENS et al.), 7 March 2002 (07.03.2002); para [0007], [0020]	12-21, 25-26
Y	WEIDE et al., Secretion of functional human enzymes by Tetrahymena thermophila. BMC Biotechnology 2006, vol 6, no 19, pp 1-9; pg 2, right col, para 1; pg 4, fig 2a; pg 7-8, Generation and transformation of expression plasmids; pg 8, Immunoprecipitation of DNase I from T. thermophila supernatants	1-21, 25-26
Y	CHILCOAT et al., Granule lattice protein 1 (Gr1p), an acidic, calcium-binding protein in Tetrahymena thermophila dense-core secretory granules, influences granule size, shape, content organization, and release but not protein sorting or condensation. The Journal of Cell Biology, 1996, vol 135, no 6, prt 2, pp 1775-1787; abstract, pg 1776, Protein Purification and Sequencing, left col, para 4; pg 1778-1779, Sequence Analysis	1-21
Y	VERBSKY et al., Proteolytic Processing and Ca21-binding Activity of Dense-Core Vesicle Polypeptides in Tetrahymena. Molecular Biology of the Cell, 1998, vol 9, pp 497-511; pg 499, para 6; pg 502, fig 3; pg 502-505, Granule Proproteins Are Processed at Multiple Conserved Sites	1-2, 12-14



Further documents are listed in the continuation of Box C.



* 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

17 August 2010 (17.08.2010)

Date of mailing of the international search report

07 SEP 2010

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

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/28165

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.: 22-24
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-21, 25, and 26, drawn to methods for production of a heterologous soluble polypeptide by a ciliate, a nucleic acid having a sequence encoding a fusion protein, and a transgenic ciliate comprising a heterologous polypeptide linked to a mucocyst-targeting polypeptide.

Group II: Claims 27-31, drawn to protein preparations and vaccine preparations.

The groups listed above 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.

See Supplemental 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 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.: 1-21, 25-26

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/US 10/28165

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BECKER et al., The Secretory Pathway of Protists: Spatial and Functional Organization and Evolution. MICROBIOLOGICAL REVIEWS, 1996, vol 60, no 4, pp 697-721; pg 699, left col para 3; pg 707, right col, para 3	11, 18-21
A	BOWMAN et al., Genomic and Proteomic Evidence for a Second Family of Dense Core Granule Cargo Proteins in Tetrahymena thermophila. J. Eukaryot. Microbiol., 2005, vol 52, no 4, pp 291-297; abstract	7, 16
A	ARREGUI et al. Ciliate contributions to bioaggregation: laboratory assays with axenic cultures of Tetrahymena thermophila"; International Microbiology(2007) Vol 10 Pages 91-96, especially pg 91, col 1	1-21, 25-26
A	US 2005/0106164 A1 (GAERTIG et al.) 19 May 2005 (19.05.2005) para [0060]; [0080]; claim 9	1-21, 25-26

Supplemental Sheet
Continuation of Box III.

Groups I and II share the technical feature of a an extracellular matrix formed by secretion by ciliates and a fusion protein encoded by said ciliates. However, this does not represent an improvement over the prior art. Gaertig et al. (US 2005/0106164 A1) teach a fusion protein encoded by a ciliate (Tetrahymena) which is secreted by the ciliate (para [0060]; [0080]; claim 9). Although Gaertig is silent with respect to a secreted extracellular matrix, this limitation is either inherent in the teachings of Gaertig (note that Gaertig teaches expression of proteins in secretory mucocysts; para [0101]) or it would have obvious to one skilled in the art because Arregui ("Ciliate contributions to bioaggregation: laboratory assays with axenic cultures of Tetrahymena thermophila"; INTERNATIONAL MICROBIOLOGY (2007) 10:91-96) teaches The main step in the development of microbial consortia is the formation of an extracellular matrix, which is produced by the biological activity of the bacteria , protists (e.g.Tetrahymena; abstract) , and other organisms that support the structure of the bioaggregates (pg 91, col 1).

Accordingly, unity of invention is lacking.