

(51) International Patent Classification:
C12N 5/10 (2006.01)(21) International Application Number:
PCT/US2011/045316(22) International Filing Date:
25 July 2011 (25.07.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
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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, 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, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, 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, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:
5 April 2012

(54) Title: VECTORS AND METHODS FOR RECOMBINANT PROTEIN EXPRESSION

General configuration

One promoter with IRES for two coding sequences

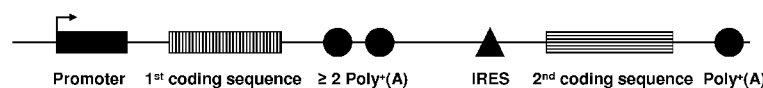


Figure 1

1st and 2nd coding sequences encode for recombinant proteins
 2nd coding sequence also encodes for selection marker
 IRES represents IRES or its mutant

(57) **Abstract:** The present invention discloses a series of eukaryotic expression vectors utilizing the reduction of transcription read-through events to create stable and high-yield cell lines for recombinant protein expression. The vectors comprise more than one polyadenylation signal or one or more polyadenylation signals plus other DNA fragment which is known to enhance transcription termination to control the expression level of selection marker, with the configuration to transcribe the minimal level of full-length bicistronic mRNA to express the selection marker, which can be used to create stable cell lines at high expression levels, without the need for drug selection or drug mediated gene amplification.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/45316

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/00 (2011.01)

USPC - 435/320.1

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) - C12N 15/00 (2011.01)

USPC - 435/320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 536/23.1Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB), Google Scholar: Internal ribosome entry site, IRES, signal, sequence, polyadenylation, polyA, polyaden\$, terminat\$, selec\$, marker, vector, expres\$, cell, cellular, system, eukar\$, mamm\$, antibod\$, antibi\$, resist\$, neomycin, puromycin, hygromycin, dihydrofolate reductase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0210952 A1 (WU et al.) 20 August 2009 (20.08.2009) para [0010]; [0085]-[0088]; [0099]-[0100]; [0120]; [0127]; [0155]-[0156]; [0164]; [0186]; [0211]; [0220]; [0228]; [0243]; [0314]; [0326]	1-7, 9-16
A	US 2009/0162900 A1 (ENENKEL et al.) 25 June 2009 (25.06.2009) para [0072]; [0075]-[0076]; [0079]; [0081]-[0086]; [0088]-[0089]	1-7, 9-16

☐ 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

05 December 2011 (05.12.2011)

Date of mailing of the international search report

16 DEC 2011

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

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PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/45316

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.: 8
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:
Claim 8 is unsearchable because Applicant failed to submit a valid CRF to the ISA/225 of 16 August 2011.

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 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.:

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.