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Declarations under Rule 4.17:

- 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))

[Continued on next page]

(54) Title: PROMOTERS FOR HIGH LEVEL RECOMBINANT EXPRESSION IN FUNGAL HOST CELLS

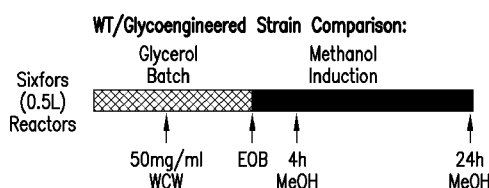


FIG. 1A

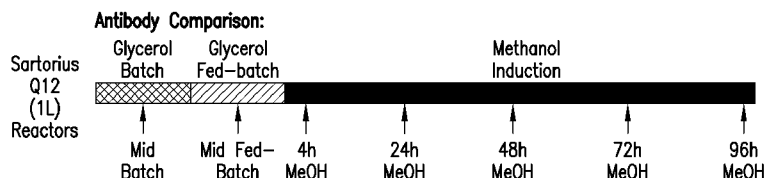


FIG. 1B

(57) Abstract: The present invention provides, in part, promoters for recombinant expression of polypeptides in host cells such as *Pichia pastoris* as well as methods of use thereof. In one embodiment, an isolated hybrid polynucleotide comprises a promoter selected from a group of *Pichia pastoris* promoters. In another embodiment, a method for making a polypeptide by introducing an isolated hybrid polynucleotide into an isolated fungal host cell while utilizes a *Pichia pastoris* promoter.



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3 January 2014

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/029146

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 1/19 (2012.01)

USPC - 526/24.1

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 1/19, 15/00, 09; C12P 21/02, 04 (2012.01)

USPC - 435/69.1, 254.2, 254.23, 320.1; 526/24.1

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)

PatBase, Google Patents, PubMed, NCBI BLAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RUTH et al. Variable production windows for porcine trypsinogen employing synthetic inducible promoter variants in Pichia pastoris. Syst. Syth. Biol. 4:181-191. 29 May 2010. entire document	1-11
A	US 6,258,559 B1 (ZAMOST) 10 July 2001 (10.07.2001) entire document	1-11
A	US 2010/0196913 A1 (HARTNER et al) 05 August 2010 (05.08.2010) entire document	1-11

☐ 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

14 August 2012

Date of mailing of the international search report

04 SEP 2012

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/029146

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.: 12, 13
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:
Claims 12 and 13 are unsearchable due to the elected promoters in the Applicant's response to unity of invention not being present in the claims.

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:

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 need to be paid.

Group I+: claims 1-13 are drawn to an isolated hybrid polynucleotide comprising a promotor and methods of making the same.

The first invention of Group I+ is restricted to the isolated hybrid polynucleotide wherein the promotor is selected to be *Pichia pastoris* Pp01g09290 (ScFBA1) promotor. Should an additional fee(s) be paid, Applicant is invited to elect additional promotors to be searched. The exact claims searched will depend on Applicant's election of specific promotors. The inventions listed in Groups I+ 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:

The Group I+ promotors do not share a significant structural element and further include the alternative selection of any known promotor that is "a functional variant thereof."

Group I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

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.:
Claims 1-13 as related to PpPIR1, ScCHT2, ScAAC2/PET9, and ScPST1

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.