PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 93331-920858	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2014/064168	International filing date *(day/month/year)* 05 November 2014 (05.11.2014)	Priority date *(day/month/year)* 05 November 2013 (05.11.2013)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 10 May 2016 (10.05.2016)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Simin Baharlou e-mail: pt09.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: KEVIN M. CLARK
KILPATRICK TOWNSEND & STOCKTON LLP
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SAN FRANCISCO, CA 94111

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	04 FEB 2015
Applicant's or agent's file reference 93331-920858	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 14/64168	05 November 2014 (05.11.2014)	05 November 2013 (05.11.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C07K 14/40, C12N 1/19, C12N 15/09, C12P 7/10 (2015.01)
CPC - C07K 14/40, Y02E 50/16

Applicant BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	21 January 2015 (21.01.2015)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-36	YES
	Claims	None	NO
Inventive step (IS)	Claims	1-36	YES
	Claims	None	NO
Industrial applicability (IA)	Claims	1-36	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-8, 16, 18, 20-25, 35 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest (claim 1) a recombinant xylose transporter protein comprising a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41 of Candida intermedia GXS1 protein, wherein said transporter motif sequence is -G-G/F-X1-X2-X3-G-; wherein, X1 is D, C, G, H, I, L, or F; X2 is A, D, C, E, G, H, or I; X3 is N, C, Q, F, G, L, M, S, T, or P; and wherein, said transporter motif sequence is not -G-G-L-I-F-G- or-G-G-F-I-F-G-.

Regarding claim 1, the article entitled "A molecular transporter engineering approach to improving xylose catabolism in Saccharomyces cerevisiae" by Young et al. (hereinafter 'Young 2012') discloses a recombinant xylose transporter protein comprising S at amino acid residue position 40 (i.e. position X3) of Candida intermedia GXS1 protein (abstract "Here, we describe the directed evolution of two heterologous transporters, Candida intermedia GXS1 and Scheffersomyces stipitis XUT3 ... Analysis of mutations highlights several important residues influencing transporter function including point mutations at F40 of C. intermedia GXS1 ... This work is the first.to demonstrate that molecular transporter proteins can be improved for biotechnological applications through directed evolution in yeast"; p 404, col 2, para 3 "The Quikchange II kit from Stratagene was used to introduce either F40Vor F40S into the wild-type C. intermedia GXS1"). Young 2012 does not teach a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41, wherein said transporter motif sequence is -G-G/F-X1-X2-X3-G-; wherein, X1 is D, C, G, H, I, L, or F; X2 is A, D, C, E, G, H, or I; and wherein, said transporter motif sequence is not -G-G-L-I-F-G- or-G-G-F-I-F-G-.

The wild-type amino acid sequence for Candida intermedia GXS1 protein is know in the art, as evidenced by GenBank Accession No. CAI44932 (hereinafter 'CAI44932') which discloses the amino acid sequence for Candida intermedia GXS1 protein (title "glucose/xylose symporter 1 [Candida intermedia]") having the sequence of -G-G-V-L-F-G- for amino acid residue positions 36, 37, 38, 39, 40, and 41 (sequence of CAI44932, amino acids 36-41). Although Young 2012 discloses modifying the amino acid at position 40 of the GXS1 sequence from F to S (abstract "point mutations at F40 of C. intermedia GXS1"; p 404, col 2, para 3 "introduce either F40Vor F40S into the wild-type C. intermedia GXS1"), there is nothing that would lead one of ordinary skill in the art to further modify the GXS1 protein sequence of CAI44932 at positions 38 and 39 to obtain a transporter motif sequence -G-G/F-X1-X2-X3-G-; wherein, X1 is D, C, G, H, I, L, or F; X2 is A, D, C, E, G, H, or I; and wherein, said transporter motif sequence is not -G-G-L-I-F-G- or-G-G-F-I-F-G- as described in claim 1.

Another reference that teaches Candida intermedia GXS1 protein is US 2011/0020910 A1 to Glass et al. (hereinafter 'Glass'), which discloses recombinant sugar transporters that can include Gxs1 from C. intermedia (para [0225] "In certain embodiments of the invention, the host cell contains a recombinant polynucleotide encoding a pentose transporter. ... pentose transporters may include, Gxs1 from C. intermedia") and methods of increasing transport of xylose into a cell by providing a host cell with a recombinant polynucleotide encoding a polypeptide that results in increased transport of xylose into the cell (para [0053] "methods of increasing transport of xylose, arabinose, or glucose into a cell, including providing a host cell, where the host cell contains a recombinant polynucleotide ... where expression of the recombinant polynucleotide results in increased transport of xylose, arabinose, or glucose into the cell compared with a cell that does not contain the recombinant polynucleotide"). However, Glass also does not teach a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41 of the GXS1 protein, wherein said transporter motif sequence is -G-G/F-X1-X2-X3-G-; wherein, X1 is D, C, G, H, I, L, or F; X2 is A, D, C, E, G, H, or I; X3 is N, C, Q, F, G, L, M, S, T, or P; and wherein, said transporter motif sequence is not -G-G-L-I-F-G- or-G-G-F-I-F-G-.

A further reference that teaches Candida intermedia GXS1 protein is WO 2012/097091 A2 to Salmon et al. (hereinafter 'Salmon'), which discloses sugar transporter protein of Candida intermedia GXS1 protein, wherein a microorganism such as yeast can be engineered to add, amplify, or increase the activity of sugar transport systems by increasing the number of GXS1 genes (p 56, ln 28 -- p 57, ln 4). However, Salmon also does not teach a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41 of the GXS1 protein, wherein said transporter motif sequence is -G-G/F-X1-X2-X3-G-; wherein, X1 is D, C, G, H, I, L, or F; X2 is A, D, C, E, G, H, or I; X3 is N, C, Q, F, G, L, M, S, T, or P; and wherein, said transporter motif sequence is not -G-G-L-I-F-G- or-G-G-F-I-F-G-.

---------Please see continuation in supplemental box---------

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INTERNATIONAL SEARCHING AUTHORITY

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V2 Citations and Explanations

The article entitled "Rewiring yeast sugar transporter preference through modifying a conserved protein motif" by Young et al. (hereinafter 'Young 2014') discloses a recombinant xylose transporter protein comprising a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41 of Candida intermedia GXS1 protein (abstract; p 131, col 2, para 2 "we report on the discovery of a conserved Gly36-Gly37-Val38-Leu39-Phe40-Gly41 motif surrounding the previously identified Phe40 residue of C. intermedia GXS1 ... We conduct saturation mutagenesis on Val38, Leu39, and Phe40 within the variable region of this motif in C. intermedia GXS1 to demonstrate control of sugar selectivity"), wherein said transporter motif sequence is -G-G/F-X1-X2-X3-G- (p 131, col 2, para 2 "G-G/F-XXX-G");
wherein, X1 is D, C, G, H, I, L, or F (p 132, col 2, para 3 -- p 133, col 1, para 1; p 133, Fig 3A, showing substitution of amino acid residue Val at position 38 for Ala, Arg, Asn, Asp, Cys, Gln, Blu, Bly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val);
X2 is A, D, C, E, G, H, or I (p 133, col 2, para 2; p 133, Fig 3B, showing substitution of amino acid residue Leu at position 39 for Ala, Arg, Asn, Asp, Cys, Gln, Blu, Bly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val);
X3 is N, C, Q, F, G, L, M, S, T, or P (p 133, col 2, para 3; p 133, Fig 3C, showing substitution of amino acid residue Phe at position 40 for Ala, Arg, Asn, Asp, Cys, Gln, Blu, Bly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val); and
wherein, said transporter motif sequence is not -G-G-L-I-F-G- or-G-G-F-I-F-G- (p 132, col 2, para 3 -- p 133, col 2, para 3; p 133, Fig 3A, 3B, and 3C, showing mutant motifs combinations need not be -G-G-L-I-F-G- or-G-G-F-I-F-G-).
However, Young 2014 was published on 7 January 2014 and is therefore not prior art.

There is no prior art that teaches or fairly suggests a recombinant xylose transporter protein comprising the modified transporter motif sequence described in claim 1 and therefore claim 1 meets the criteria set out in PCT Article 33(2)-(3).

Regarding claims 2-8, 16, 18, 20-25, and 35, the claims are dependent upon claim 1 and therefore meet the criteria for substantially the same reasons.

Claims 9-15, 17, 19, 26-34, 36 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest (claim 9) a recombinant galactose-arabinose transporter protein comprising a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41 of Candida intermedia GXS1 protein, wherein said transporter motif sequence is -G-G/F-X4-X5-X6-G-; wherein, X4 is D, C, F, G, H, L, R, T, or P; X5 is A, C, E, F, H, K, S, P, or V; X6 is R, D, E, F, H, I, M, T, or Y; and wherein said sequence is not -G-G-L-V-Y-G-, or -G-G-F-V-F-G-.

Regarding claim 9, Young 2012 discloses a recombinant xylose transporter protein comprising amino acid residue position 40 of Candida intermedia GXS1 protein (abstract). Young 2012 does not teach a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41, wherein said transporter motif sequence is -G-G/F-X4-X5-X6-G-; wherein, X4 is D, C, F, G, H, L, R, T, or P; X5 is A, C, E, F, H, K, S, P, or V; X6 is R, D, E, F, H, I, M, T, or Y; and wherein said sequence is not -G-G-L-V-Y-G-, or -G-G-F-V-F-G-.

The amino acid sequence for Candida intermedia GXS1 protein is know in the art, as evidenced by CAI44932 which discloses the amino acid sequence for Candida intermedia GXS1 protein (title "glucose/xylose symporter 1 [Candida intermedia]") having the sequence of -G-G-V-L-F-G- for amino acid residue positions 36, 37, 38, 39, 40, and 41 (sequence of CAI44932, amino acids 36-41). Although Young 2012 discloses modifying the amino acid at position 40 of the GXS1 sequence (abstract "point mutations at F40 of C. intermedia GXS1"; p 404, col 2, para 3 "introduce either F40Vor F40S into the wild-type C. intermedia GXS1"), there is nothing that would lead one of ordinary skill in the art to further modify the GXS1 protein sequence of CAI44932 at positions 38, 39, and 40 to obtain a transporter motif sequence -G-G/F-X4-X5-X6-G-; wherein, X4 is D, C, F, G, H, L, R, T, or P; X5 is A, C, E, F, H, K, S, P, or V; X6 is R, D, E, F, H, I, M, T, or Y; and wherein said sequence is not -G-G-L-V-Y-G-, or -G-G-F-V-F-G- as described in claim 9.

Another reference that teaches Candida intermedia GXS1 protein is Glass, which discloses recombinant sugar transporters that can include Gxs1 from C. intermedia (para [0225] "In certain embodiments of the invention, the host cell contains a recombinant polynucleotide encoding a pentose transporter. ... pentose transporters may include, Gxs1 from C. intermedia") and methods of increasing transport of arabinose into a cell by providing a host cell with a recombinant polynucleotide encoding a polypeptide that results in increased transport of arabinose into the cell (para [0053]). However, Glass also does not teach a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41 of the GXS1 protein, wherein said transporter motif sequence is -G-G/F-X4-X5-X6-G-; wherein, X4 is D, C, F, G, H, L, R, T, or P; X5 is A, C, E, F, H, K, S, P, or V; X6 is R, D, E, F, H, I, M, T, or Y; and wherein said sequence is not -G-G-L-V-Y-G-, or -G-G-F-V-F-G-.

A further reference that teaches Candida intermedia GXS1 protein is Salmon, which discloses sugar transporter protein of Candida intermedia GXS1 protein, wherein a microorganism such as yeast can be engineered to add, amplify, or increase the activity of sugar transport systems by increasing the number of GXS1 genes (p 56, ln 28 -- p 57, ln 4). However, Salmon also does not teach a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41 of the GXS1 protein, wherein said transporter motif sequence is -G-G/F-X4-X5-X6-G-; wherein, X4 is D, C, F, G, H, L, R, T, or P; X5 is A, C, E, F, H, K, S, P, or V; X6 is R, D, E, F, H, I, M, T, or Y; and wherein said sequence is not -G-G-L-V-Y-G-, or -G-G-F-V-F-G-.

--------Please see continuation in next supplemental box--------

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 14/64168

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V2 Citations and Explanations

Young 2014 discloses a recombinant xylose transporter protein comprising a transporter motif sequence corresponding to amino acid residue positions 36, 37, 38, 39, 40, and 41 of Candida intermedia GXS1 protein (abstract; p 131, col 2, para 2 "we report on the discovery of a conserved Gly36-Gly37-Val38-Leu39-Phe40-Gly41 motif surrounding the previously identified Phe40 residue of C. intermedia GXS1 ... We conduct saturation mutagenesis on Val38, Leu39, and Phe40 within the variable region of this motif in C. intermedia GXS1 to demonstrate control of sugar selectivity"),
wherein said transporter motif sequence is -G-G/F-X4-X5-X6-G- (p 131, col 2, para 2 "G-G/F-XXX-G");
wherein, X4 is D, C, F, G, H, L, R, T, or P (p 132, col 2, para 3 -- p 133, col 1, para 1; p 133, Fig 3A, showing substitution of amino acid residue Val at position 38 for Ala, Arg, Asn, Asp, Cys, Gln, Blu, Bly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val);
X5 is A, C, E, F, H, K, S, P, or V (p 133, col 2, para 2; p 133, Fig 3B, showing substitution of amino acid residue Leu at position 39 for Ala, Arg, Asn, Asp, Cys, Gln, Blu, Bly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val);
X6 is R, D, E, F, H, I, M, T, or Y (p 133, col 2, para 3; p 133, Fig 3C, showing substitution of amino acid residue Phe at position 40 for Ala, Arg, Asn, Asp, Cys, Gln, Blu, Bly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val); and
wherein said sequence is not -G-G-L-V-Y-G-, or -G-G-F-V-F-G- (p 132, col 2, para 3 -- p 133, col 2, para 3; p 133, Fig 3A, 3B, and 3C, showing mutant motifs combinations need not be -G-G-L-V-Y-G- or -G-G-F-V-F-G-).
However, Young 2014 was published on 7 January 2014 and is therefore not prior art.

There is no prior art that teaches or fairly suggests a recombinant xylose transporter protein comprising the modified transporter motif sequence described in claim 9 and therefore claim 9 meets the criteria set out in PCT Article 33(2)-(3).

Regarding claims 10-15, 17, 19, 26-34, and 36, the claims are dependent upon claim 9 and therefore meet the criteria for substantially the same reasons.

Claims 1-36 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.