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*[Continued on next page]*

(54) Title: ZYMOMONAS WITH IMPROVED XYLOSE UTILIZATION

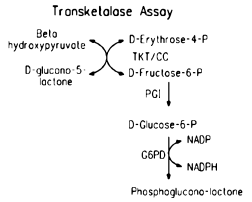


FIG. 1A

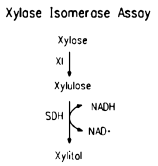


FIG. 1C

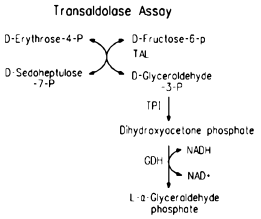


FIG. 1B

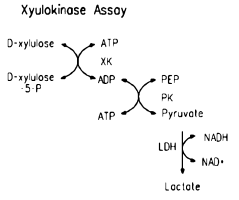


FIG. 1D

(57) Abstract: Strains of *Zymomonas* were engineered by introducing a chimeric xylose isomerase gene that contains a mutant promoter of the *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene. The promoter directs increased expression of xylose isomerase, and when the strain is in addition engineered for expression of xylulokinase, transaldolase and transketolase, improved utilization of xylose is obtained.

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# TITLE

# ZYMOMONAS WITH IMPROVED XYLOSE UTILIZATION

## CROSS-REFERENCE TO RELATED APPLICATION

**[0001]** This application claims the benefit of U.S. Provisional Application No. 61/039878 filed on March 27, 2008, which application is incorporated herein by reference.

## STATEMENT OF GOVERNMENT RIGHTS

**[0002]** This invention was made with United States government support under Contract Nos. 04-03-CA-70224 and DE-FC36-03GO13146 awarded by the Department of Energy. The United States government has certain rights in this invention. Further, the United States Government has rights in this invention under Contract No. DE-AC36-99GO10337 between the United States Department of Energy and the National Renewable Energy Laboratory, a Division of the Midwest Research Institute.

## FIELD OF INVENTION

**[0003]** The invention relates to the fields of microbiology and genetic engineering. More specifically, genetic engineering of *Zymomonas* strains with improved xylose utilization is described.

## BACKGROUND OF INVENTION

**[0004]** Production of ethanol by microorganisms provides an alternative energy source to fossil fuels and is therefore an important area of current research. It is desirable that microorganisms producing ethanol, as well as other useful products, be capable of using xylose as a carbon source since xylose is the major pentose in hydrolyzed lignocellulosic materials, and therefore can provide an abundantly available, low cost carbon substrate. *Zymomonas mobilis* and other bacterial ethanologens which do not naturally utilize xylose may be genetically engineered for xylose utilization by introduction of genes encoding 1) xylose isomerase, which catalyses the conversion of xylose to xylulose; 2) xylulokinase, which phosphorylates xylulose to form xylulose 5-phosphate; 3) transketolase; and 4) transaldolase.

**[0005]** There has been success in engineering *Z. mobilis* strains for xylose metabolism (US 5514583, US 5712133, US 6566107, WO 95/28476, Feldmann et al. (1992) Appl Microbiol Biotechnol 38: 354-361, Zhang et al. (1995) Science 267:240-243), as well as a *Zymobacter palmae* strain (Yanase et al. (2007) Appl. Environ. Mirobiol. 73:2592-2599). However, typically the engineered strains do not grow and produce ethanol as well on xylose as on glucose. Strains engineered for xylose utilization have been adapted by serial passage on xylose medium, resulting in strains with improved xylose utilization as described in U. S. Pat. 7,223,575 and commonly owned and co-pending US Patent App. Pulbication No. US20080286870. However the genetic basis for the improvement had not been determined.

**[0006]** There remains a need for genetically engineered strains of *Zymomonas*, and other bacterial ethanolagens, having improved xylose utilization. Applicants have discovered genetic alterations of *Z. mobilis* strains engineered for xylose utilization and adapted for improved xylose utilization, and used the discovery to engineer strains for improved xylose utilization.

## SUMMARY OF INVENTION

**[0007]** The present invention relates to strains of bacteria that are genetically engineered for xylose utilization by transforming with a chimeric gene encoding xylose isomerase that is expressed from an improved *Zymomonas mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter (P*gap*). The strains are also transformed with genes for expression of xylulokinase, transaldolase and transketolase. The improved P*gap* directs higher expression than the native P*gap* which causes improved xylose utilization as compared to strains not having an improved P*gap* for expression of xylose isomerase.

**[0008]** Described herein is a recombinant bacterial strain selected from the group consisting of *Zymomonas* and *Zymobacter* comprising a gene introduced by transformation, the gene comprising:
a) an isolated nucleic acid molecule comprising a *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter that has a base substitution in a position selected from the group consisting of position -190, position -89, or both position -190 and -89; wherein the position numbers are with respect to the natural ATG translation initiation codon for glyceraldehyde-3-phosphate dehydrogenase in the CP4 and ZM4 strains of *Z. mobilis*; which is an improved P*gap*; and
b) an operably linked isolated nucleic acid molecule encoding xylose isomerase.
The gene introduced by the transformation steps above may be a chimeric gene comprising the mutations for enhanced expression of P*gap*.

**[0009]** Also described herein is a process for engineering a bacterial strain selected from the group consisting of *Zymomonas* and *Zymobacter* comprising transforming with a gene, e.g. a chimeric gene comprising;

a) an isolated nucleic acid molecule comprising a *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter that has a base substitution in a position selected from the group consisting of position -190, position -89, or both position -190 and -89; wherein the position numbers are with respect to the natural ATG translation initiation codon for glyceraldehyde-3-phosphate dehydrogenase in the CP4 and ZM4 strains of *Z. mobilis*; which is an improved P*gap*; and

b) an operably linked isolated nucleic acid molecule encoding a xylose isomerase enzyme.

**[00010]** Another process described herein is for engineering a xylose-utilizing bacterial strain selected from the group consisting of *Zymomonas* and *Zymobacter* comprising in any order the steps of:

a) transforming with genes or an operon for expression of transaldolase and transketolase; and
b) transforming with genes or an operon for expression of xylose isomerase and xylulokinase, wherein the xylose isomerase enzyme is expressed from a *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter that has a base substitution in a position selected from the group consisting of position -190, position -89, or both position -190 and -89; wherein the position numbers are with respect to the natural ATG translation initiation codon for glyceraldehyde-3-phosphate dehydrogenase in the CP4 and ZM4 strains of *Z. mobilis*; which is an improved P*gap;*

.

**[0010]** Also described herein is a process for production of ethanol from a medium comprising xylose, comprising culturing in the medium a recombinant bacterial strain selected from the group consisting of *Zymomonas* and *Zymobacter* comprising a chimeric gene introduced by transformation, the chimeric gene comprising:
a) an isolated nucleic acid molecule comprising a *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter that has a base substitution in a position selected from the group consisting of position -190, position -89, or both position -190 and -89; wherein the position numbers are with respect to the natural ATG translation initiation codon for glyceraldehyde-3-phosphate dehydrogenase in the CP4 and ZM4 strains of *Z. mobilis*; which is an improved P*gap*; and
b) an operably linked isolated nucleic acid molecule encoding xylose isomerase.

**[0011]** In addition, a recombinant bacterial strain is describe herein that is selected from the group consisting of *Zymomonas* and *Zymobacter* and which is engineered to express xylose isomerase at a level to produce at least about 0.1 μmoles product/mg protein/minute, as determined by reacting 20 μL of cell free extract in a reaction mix, at 30°C, comprising 0.256 mM NADH, 50 mM xylose,

10 mM $MgSO_4$, 10 mM triethanolamine, and 1U/ml sorbitol dehydrogenase, wherein D-xylulose is the product.

## BRIEF DESCRIPTION OF THE FIGURES AND SEQUENCE DESCRIPTIONS

**[0012]** The various embodiments of the invention can be more fully understood from the following detailed description, the figures, and the accompanying sequence descriptions, which form a part of this application.

Figure 1 shows the strategies for enzyme assays of transketolase (A), transaldolase (B), xylose isomerase (C), and xyulokinase (D).

Figure 2 shows a graph of xylose isomerase (XI) and xylulokinase (XK) activities in T2C, T3C, T4C, and T5C lines transformed with P*gapxylAB*.

Figure 3 shows a graph of transaldolse (TAL) and transketolase (TKT) activities in T2C, T3C, T4C, and T5C lines transformed with P*gapxylAB*.

Figure 4 shows a graph of % theoretical ethanol yield and % xylose utilization of selected adapted xylose-utilizing strain colonies.

Figure 5 shows a graph of growth of adapted xylose-utilizing strains at 70 hr on RM (rich medium) with 5% xylose (RMX5%) before and after growing 50 generations in RM with 5% glucose (RMG).

Figure 6 shows plasmid maps of (A) pZB188; (B) pZB188/aadA; and (C) pZB188/aadA-GapXylA; as well as (D) a schematic representation of the *E. coli* xylose isomerase expression cassette P*gap*XylA.

Figure 7 shows plasmid maps of (A) pMOD™-2-<MCS>; (B) pMOD-Linker; and (C) pMOD-Linker-Spec.

Figure 8 shows a plasmid map of pLDHSp-9WW.

Figure 9 shows a plasmid map of pMOD-Linker-Spec-801GapXylA.

Figure 10 shows plasmid maps of (A) pMOD-Linker-Spec-801GapXylA; (B) pZB188/aadA-GapXylA; and (C) pZB188/aadA-801GapXylA.

Figure 11 shows a graph of growth curves (OD600 versus time) in xylose-containing media for the three strains that harbored the P*gap-E. coli* xylose isomerase expression plasmid (X1, X2 and X2) and the three strains that harbored the control plasmid (C1, C2 and C3).

Figure 12 shows graphs of growth curves (OD600 versus time) of strains ZW641, ZW658, X1 and C1 in xylose-containing media without spectinomycin plotted in (A) on a linear scale, and in (B) on a logarithmic scale.

Figure 13 shows graphs of growth curves (OD600 versus time) of three strains with integrated 801P*gap*-XylA (#8-2, #8-4, #8-5) and of three strains with integrated 641P*gap*-XylA (#6-1, #6-3, #6-5) compared to strain ZW658, plotted in (A) on a linear scale, and in (B) on a logarithmic scale.

Table 3 is a table of the Profile HMM for xylose isomerases. Table 3 is submitted herewith electronically and is incorporated herein by reference.

**[0013]** The invention can be more fully understood from the following detailed description and the accompanying sequence descriptions which form a part of this application.

The following sequences conform with 37 C.F.R. 1.821-1.825 ("Requirements for Patent Applications Containing Nucleotide Sequences and/or Amino Acid Sequence Disclosures - the Sequence Rules") and are consistent with World Intellectual Property Organization (WIPO) Standard ST.25 (1998) and the sequence listing requirements of the EPO and PCT (Rules 5.2 and 49.5(a-bis), and Section 208 and Annex C of the Administrative Instructions). The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

SEQ ID NO:1 is the nucleotide sequence of the *Zm*P*gap* from the CP4 strain of Z. mobilis.

SEQ ID NO:2 is the nucleotide sequence of the *Zm*P*gap* from the ZM4 strain of Z. mobilis.

SEQ ID NO:3 is the nucleotide sequence of the *Zm*P*gap* from pZB4, which is also in the P*gapxylAB* operon of strains ZW641 and 8XL4.

SEQ ID NO:4 is the nucleotide sequence of the improved P*gap* from strain ZW658.

SEQ ID NO:5 is the nucleotide sequence of the improved P*gap* from strain 8b.

SEQ ID NO:6 is the nucleotide sequence of an improved P*gap* with both -190 (ZW658) and -89 (8b) mutations in the pZB4 variant of P*gap*.

SEQ ID NO:7 is the nucleotide sequence of an improved P*gap* with the -190 mutation from ZW658 in the CP4 variant of P*gap*.

SEQ ID NO:8 is the nucleotide sequence of an improved P*gap* with the -89 mutation from 8b in the CP4 variant of P*gap*.

SEQ ID NO:9 is the nucleotide sequence of an improved P*gap* with both -190 (ZW658) and -89 (8b) mutations in the CP4 variant of P*gap*.

SEQ ID NO:10 is the nucleotide sequence of an improved P*gap* with the -190 mutation from ZW658 in the ZM4 variant of P*gap*.

SEQ ID NO:11 is the nucleotide sequence of an improved P*gap* with the -89 mutation from 8b in the ZM4 variant of P*gap*.

SEQ ID NO:12 is the nucleotide sequence of an improved P*gap* with both -190 (ZW658) and -89 (8b) mutations in the ZM4 variant of P*gap*.

SEQ ID NOs:13 and 14 are the nucleotide sequences of primers for amplification of a DNA fragment containing the glyceraldehyde-3-phosphate dehydrogenase gene promoter (P*gap*) from pZB4.

SEQ ID NOs:15 and 16 are the nucleotide sequences of primers for amplification of a DNA fragment containing a *tal* coding region from pZB4.

SEQ ID NOs:17 and 18 are the nucleotide sequences of primers for amplification of a DNA fragment containing P*gaptal* from the P*gap* and *tal* fragments.

SEQ ID NOs:19 and 20 are the nucleotide sequences of primers for amplification of a DNA fragment containing *loxP*::Cm from pZB186.

SEQ ID NO:21 is the complete nucletotide sequence for the pMODP*gaptaltkt*Cm plasmid.

SEQ ID NOs:22 and 23 are the nucleotide sequences of primers for amplification of a 3 kb DNA fragment containing *tal* and *tkt* coding regions in transformants receiving pMODP*gaptaltkt*Cm.

SEQ ID NO:24 is the complete nucletotide sequence for the pMODP*gapxylAB*Cm plasmid.

SEQ ID NOs:25 and 26 are the nucleotide sequences of primers for amplification of a 1.6 kb *PgapxylA* DNA fragment from the T2C, T3C, T4C and T5C integrants with pMODP*gapxylAB*Cm.

SEQ ID NOs:27 and 28 are the nucleotide sequences of primers for amplification of a DNA fragment containing the P*gap* from ZW641 and ZW658.

SEQ ID NOs:29-31 are the nucletotide sequences for primers for sequencing the P*gap* from ZW641 and ZW658.

SEQ ID NOs:32 and 33 are the nucleotide sequences of primers for amplification of a DNA fragment containing a Spec$^r$-cassette.

SEQ ID NO:34 is the complete nucletotide sequence of the xylose isomerase expression cassette P*gapXylA*.

SEQ ID NOs:35 and 36 are the nucleotide sequences of oligonucleotides used to substitute a different multi-cloning site in pMOD2-<MCS>.

SEQ ID NOs:37 and 38 are the nucleotide sequences of primers for amplification of the P*gapxylA* regions from strains ZW801-4 and ZW641 for insertion into pMOD-Linker-Spec to yield plasmids pMOD-Linker-Spec-801GapXylA and pMOD-Linker-Spec-641GapXylA, respectively.

SEQ ID NOs:39 and 40 are the nucleotide sequences of primers for amplification of a DNA fragment containing the P*gap* from 8XL4 and 8b.

SEQ ID NO:41 is the complete nucletotide sequence of a primer for sequencing the P*gap* from 8XL4 and 8b.

Table 1

Summary of protein and coding region SEQ ID Numbers for xylose isomerases

| Description | SEQ ID NO: Peptide | SEQ ID NO: Coding region |
|---|---|---|
| Xylose isomerase from *Escherichia coli K12* | 42 | 43 |
| Xylose isomerase from *Lactobacillus brevis* | 44 | 45 |

| | | |
|---|---|---|
| ATCC 367 | | |
| Xylose isomerase from *Thermoanaerobacterium* | 46 | 47 |
| Xylose isomerase from *Clostridium thermosulfurogenes* | 48 | 49 |
| Xylose isomerase from *Actinoplanes Missouriensis* | 50 | 51 |
| Xylose isomerase from *Arthrobacter* Strain B3728 | 52 | 53 |
| Xylose isomerase from *Bacillus licheniformis* ATCC 14580 | 54 | 55 |
| Xylose isomerase from *Geobacillus stearothermophilus* | 56 | 57 |
| Xylose isomerase from *Bacillus coagulans* 36D1 | 58 | 59 |
| Xylose isomerase from *Bacillus subtilis* subsp. subtilis str. 168 | 60 | 61 |
| Xylose isomerase from *Bacteroides vulgatus* ATCC 8482 | 62 | 63 |
| Xylose isomerase from *Bifidobacterium adolescentis* ATCC 15703 | 64 | 65 |
| Xylose isomerase from *Erwinia carotovora* subsp. atroseptica SCRI1043 | 66 | 67 |
| Xylose isomerase from *Hordeum vulgare* subsp. Vulgare | 68 | 69 |
| Xylose isomerase from *Klebsiella pneumoniae* subsp. pneumoniae MGH 78578 | 70 | 71 |
| Xylose isomerase from *Lactococcus lactis* subsp. Lactis | 72 | 73 |
| Xylose isomerase from *Lactobacillus reuteri* 100-23 | 74 | 75 |
| Xylose isomerase from *Leuconostoc mesenteroides* subsp. mesenteroides ATCC | 76 | 77 |

| | | |
|---|---|---|
| 8293 | | |
| Xylose isomerase from *Thermoanaerobacterium Thermosulfurigenes* | 78 | 79 |
| Xylose isomerase from *Thermotoga Neapolitana* | 80 | 81 |
| Xylose isomerase from *Streptomyces Rubiginosus* | 82 | 83 |
| Xylose isomerase from *Streptomyces albus* | 84 | 85[1] |
| Xylose isomerase from *Thermus thermophilus* | 86 | 87 |
| Xylose isomerase from *Streptomyces diastaticus* | 88 | 89 |
| Xylose isomerase from *Streptomyces coelicolor* A3(2) | 90 | 91 |
| Xylose isomerase from *Thermus Caldophilus* | 92 | 93[2] |
| Xylose isomerase from *Xanthomonas campestris* pv. vesicatoria str. 85-10 | 94 | 95 |
| Xylose isomerase from *Thermus aquaticus* | 96 | 97[3] |
| Xylose isomerase from *Tetragenococcus halophilus* | 98 | 99 |
| Xylose isomerase from *Staphylococcus xylosus* | 100 | 101 |
| Xylose isomerase from *Mycobacterium smegmatis* str. MC2 155 | 102 | 103 |
| Xylose isomerase from *Piromyces* sp. E2 | 104 | 105 |

[1]This coding sequence is designed, based on the *Streptomyces rubiginosus* coding sequence, to encode the *Streptomyces albus* protein (which has three amino acid differences with the *Streptomyces rubiginosus* protein.

[2]This coding sequence is designed, based on a *Thermus thermophilus* coding sequence, to encode the *Thermus Caldophilus* protein (which has 21 amino acid differences with the *Streptomyces rubiginosus* protein.

[3]This coding sequence is from *Thermus thermophilus* and translates to the *Thermus aquaticus* protein, although the *Thermus aquaticus* coding sequence may have differences due to codon degeneracy.

Table 2

Summary of Gene and Protein SEQ ID Numbers

for xylose utilization

| Description | SEQ ID NO: Peptide | SEQ ID NO: Coding region |
|---|---|---|
| Xylulokinase from *E. coli* | 106 | 107 |
| Xylulokinase from *Pseudomonas putida* W619 | 108 | 109 |
| Xylulokinase from *Rhizobium leguminosarum* bv. trifolii WSM2304 | 110 | 111 |
| Xylulokinase from *Klebsiella pneumoniae* | 112 | 113 |
| Xylulokinase from Salmonella typhimurium LT2 | 114 | 115 |
| Xylulokinase from *Rhodobacter sphaeroides* ATCC 17025 | 116 | 117 |
| transaldolase from *E. coli* | 118 | 119 |
| transaldolase from *Pseudomonas putida* W619 | 120 | 121 |
| transaldolase from *Rhizobium leguminosarum* bv. trifolii WSM2304 | 122 | 123 |
| transaldolase from *Klebsiella pneumoniae* | 124 | 125 |
| transaldolase from *Salmonella typhimurium* LT2 | 126 | 127 |
| transaldolase from *Rhodobacter sphaeroides* ATCC 17025 | 128 | 129 |
| transketolase from *E. coli* | 130 | 131 |
| transketolase from *Pseudomonas putida* W619 | 132 | 133 |
| transketolase from *Rhizobium leguminosarum* bv. trifolii WSM2304 | 134 | 135 |
| transketolase from *Klebsiella pneumoniae* | 136 | 137 |
| transketolase from *Salmonella typhimurium* LT2 | 138 | 139 |
| transketolase from *Rhodobacter sphaeroides* | 140 | 141 |

| | | |
|---|---|---|
| ATCC 17025 | | |

## DETAILED DESCRIPTION OF THE INVENTION

**[0014]** Described herein are xylose-utilizing recombinant bacterial strains that are genetically engineered to have high expression of xylose isomerase, and a process for engineering bacteria for increased xylose isomerase expression. Expression of xylose isomerase is directed by an improved *Zymomonas mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter (*Zm*P*gap*) that has at least one mutation which makes it a stronger promoter. The *Zm*P*gap* has a mutation at the -190 position, the -89 position, or both positions, with respect to the natural ATG translation initiation codon for glyceraldehyde-3-phosphate dehydrogenase in the CP4 and ZM4 strains of *Z. mobilis* (CP4 strain *Zm*P*gap*: SEQ ID NO:1 and ZM4 strain *Zm*P*gap*: SEQ ID NO:2). Xylose-utilizing recombinant bacterial strains described herein have improved fermentation on xylose-containing media. Bacteria producing ethanol or other products that are engineered as described herein may be used for increased production when grown in xylose-containing medium. For example, increased amounts of ethanol may be obtained from an ethanolagen such as *Zymomonas* that is engineered as described herein, which may be used as an alternative energy source to fossil fuels.

**[0015]** The following abbreviations and definitions will be used for the interpretation of the specification and the claims.

As used herein, the terms “comprises,” “comprising,” “includes,” “including,” “has,” “having,” “contains” or “containing,” or any other variation thereof, are intended to cover a non-exclusive inclusion. For example, a composition, a mixture, process, method, article, or apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the

contrary, “or” refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

Also, the indefinite articles “a” and “an” preceding an element or component of the invention are intended to be nonrestrictive regarding the number of instances (i.e. occurrences) of the element or component. Therefore “a” or “an” should be read to include one or at least one, and the singular word form of the element or component also includes the plural unless the number is obviously meant to be singular.

"Gene" refers to a nucleic acid fragment that expresses a specific protein or functional RNA molecule, which may include regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" or "wild type gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes.

The term "genetic construct" refers to a nucleic acid fragment that encodes for expression of one or more specific proteins or functional RNA molecules. In the gene construct the gene may be native, chimeric, or foreign in nature. Typically a genetic construct will comprise a “coding sequence”. A "coding sequence" refers to a DNA sequence that encodes a specific amino acid sequence.

"Promoter" or "Initiation control regions" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters".

The term "expression", as used herein, refers to the transcription and stable accumulation of coding (mRNA) or functional RNA derived from a gene. Expression may also refer to translation of mRNA into a polypeptide. “Antisense inhibition” refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. “Overexpression” refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. “Co-suppression” refers to the production of sense RNA transcripts or fragments capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. 5,231,020).

The term "messenger RNA (mRNA)" as used herein, refers to the RNA that is without introns and that can be translated into protein by the cell.

The term "transformation" as used herein, refers to the transfer of a nucleic acid fragment into a host organism, resulting in genetically stable inheritance. The transferred nucleic acid may be in the form of a plasmid maintained in the host cell, or some transferred nucleic acid may be integrated into the genome of the host cell. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms.

The terms "plasmid" and "vector" as used herein, refer to an extra chromosomal element often carrying genes which are not part of the central

metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "selectable marker" means an identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene's effect, i.e., resistance to an antibiotic, wherein the effect is used to track the inheritance of a nucleic acid of interest and/or to identify a cell or organism that has inherited the nucleic acid of interest.

As used herein the term "codon degeneracy" refers to the nature in the genetic code permitting variation of the nucleotide sequence without affecting the amino acid sequence of an encoded polypeptide. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

The term "codon-optimized" as it refers to genes or coding regions of nucleic acid molecules for transformation of various hosts, refers to the alteration of codons in the gene or coding regions of the nucleic acid molecules to reflect

the typical codon usage of the host organism without altering the polypeptide encoded by the DNA.

The term "*Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter" and "*Zm*P*gap*" refer to a nucleic acid molecule with promoter activity that has a base sequence that naturally occurs upstream of the glyceraldehyde-3-phosphate dehydrogenase coding region in the *Z. mobilis* genome. These terms refer to the promoters of strains of *Z. mobilis* such as the CP4 and ZM4 strains (SEQ ID NOs:1 and 2, respectively) and to variants in sequence and/or length that direct expression at a level that is not substantially different , such as the *Zm*P*gap* of pZB4 (SEQ ID NO:3).

The term "heterrologous" means not naturally found in the location of interest. For example, a heterologous gene refers to a gene that is not naturally found in the host organism, but that is introduced into the host organism by gene transfer. For example, a heterologous nucleic acid molecule that is present in a chimeric gene is a nucleic acid molecule that is not naturally found associated with the other segments of the chimeric gene, such as the nucleic acid molecules having the coding region and promoter segments not naturally being associated with each other.

As used herein, an "isolated nucleic acid molecule" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid molecule in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

A nucleic acid fragment is "hybridizable" to another nucleic acid fragment, such as a cDNA, genomic DNA, or RNA molecule, when a single-stranded form of the nucleic acid fragment can anneal to the other nucleic acid fragment under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by

reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. Stringency conditions can be adjusted to screen for moderately similar fragments (such as homologous sequences from distantly related organisms), to highly similar fragments (such as genes that duplicate functional enzymes from closely related organisms). Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45 °C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50 °C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60 °C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65 °C. An additional set of stringent conditions include hybridization at 0.1X SSC, 0.1% SDS, 65 °C and washes with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS, for example.

Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of Tm for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher Tm) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating Tm have been derived (see Sambrook et al., supra, 9.50-9.51). For hybridizations with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., supra, 11.7-11.8). In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferably a minimum

length for a hybridizable nucleic acid is at least about 15 nucleotides; more preferably at least about 20 nucleotides; and most preferably the length is at least about 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

A "substantial portion" of an amino acid or nucleotide sequence is that portion comprising enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to putatively identify that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Altschul, S. F., et al., J. Mol. Biol., 215:403-410 (1993)). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., in situ hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to specifically identify and/or isolate a nucleic acid fragment comprising the sequence. The instant specification teaches the complete amino acid and nucleotide sequence encoding particular fungal proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

The term "complementary" is used to describe the relationship between nucleotide bases that are capable of hybridizing to one another. For example,

with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine.

The terms “homology” and “homologous” are used interchangeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases do not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences.

Moreover, the skilled artisan recognizes that homologous nucleic acid sequences encompassed by this invention are also defined by their ability to hybridize, under moderately stringent conditions (e.g., 0.5 X SSC, 0.1% SDS, 60 °C) with the sequences exemplified herein, or to any portion of the nucleotide sequences disclosed herein and which are functionally equivalent to any of the nucleic acid sequences disclosed herein.

The term “percent identity”, as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, “identity” also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. “Identity” and “similarity” can be readily calculated by known methods, including but not limited to those described in: 1.) Computational Molecular Biology (Lesk, A. M., Ed.) Oxford University: NY (1988); 2.) Biocomputing: Informatics and Genome Projects (Smith, D. W., Ed.) Academic: NY (1993); 3.) Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., Eds.) Humania: NJ (1994); 4.) Sequence Analysis in Molecular Biology (von Heinje, G., Ed.) Academic (1987); and 5.) Sequence Analysis Primer (Gribskov, M. and Devereux, J., Eds.) Stockton: NY (1991).

Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the MegAlign™ program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences is performed using the "Clustal method of alignment" which encompasses several varieties of the algorithm including the "Clustal V method of alignment" corresponding to the alignment method labeled Clustal V (described by Higgins and Sharp, *CABIOS*. 5:151-153 (1989); Higgins, D.G. et al., *Comput. Appl. Biosci.,* 8:189-191 (1992)) and found in the MegAlign™ program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.). For multiple alignments, the default values correspond to GAP PENALTY=10 and GAP LENGTH PENALTY=10. Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. After alignment of the sequences using the Clustal V program, it is possible to obtain a "percent identity" by viewing the "sequence distances" table in the same program. Additionally the "Clustal W method of alignment" is available and corresponds to the alignment method labeled Clustal W (described by Higgins and Sharp, *CABIOS*. 5:151-153 (1989); Higgins, D.G. et al., *Comput. Appl. Biosci.* 8:189-191(1992)) and found in the MegAlign™ v6.1 program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.). Default parameters for multiple alignment (GAP PENALTY=10, GAP LENGTH PENALTY=0.2, Delay Divergen Seqs(%)=30, DNA Transition Weight=0.5, Protein Weight Matrix=Gonnet Series, DNA Weight Matrix=IUB ). After alignment of the sequences using the Clustal W program, it is possible to obtain a "percent identity" by viewing the "sequence distances" table in the same program.

It is well understood by one skilled in the art that many levels of sequence identity are useful in identifying polypeptides, from other species, wherein such polypeptides have the same or similar function or activity. Useful examples of percent identities include, but are not limited to: 24%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or any integer percentage from 24% to 100% may be useful in describing the present invention, such as 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. Suitable nucleic acid fragments not only have the above homologies but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids.

The term “sequence analysis software” refers to any computer algorithm or software program that is useful for the analysis of nucleotide or amino acid sequences. “Sequence analysis software” may be commercially available or independently developed. Typical sequence analysis software will include, but is not limited to: 1.) the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI); 2.) BLASTP, BLASTN, BLASTX (Altschul et al., J. Mol. Biol., 215:403-410 (1990)); 3.) DNASTAR (DNASTAR, Inc. Madison, WI); 4.) Sequencher (Gene Codes Corporation, Ann Arbor, MI); and 5.) the FASTA program incorporating the Smith-Waterman algorithm (W. R. Pearson, Comput. Methods Genome Res., [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Plenum: New York, NY). Within the context of this application it will be understood that where sequence analysis software is used for analysis, that the results of the analysis will be based on the “default values” of the program referenced, unless otherwise

specified. As used herein "default values" will mean any set of values or parameters that originally load with the software when first initialized.

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1989 (hereinafter "Maniatis"); and by Silhavy, T. J., Bennan, M. L. and Enquist, L. W. *Experiments with Gene Fusions*; Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1984; and by Ausubel, F. M. et al., In *Current Protocols in Molecular Biology*, published by Greene Publishing and Wiley-Interscience, 1987.

Discovery of Improved *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoters

**[0016]** The promoter of the *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene (*Zm*P*gap* or P*gap*) has been used for expression of chimeric genes in *Zymomonas mobilis* and *Zymobacter palmae*. When this promoter has been used to express genes for xylose metabolism, the resulting xylose utilization typically has not been as effective as desired. A recombinant *Z. mobilis* strain engineered to express the four xylose metabolism enzymes (xylose isomerase, xylulokinase, transketolase, and transaldolase) with limited xylose utilizing ability was further adapted on xylose medium for improved xylose utilization (described in commonly owned and co-pending U.S. App. Publication No. US20080286870).

**[0017]** Applicants have discovered, as described in Example 3 herein, that the improved xylose-utilizing strain called ZW658 (ATCC # PTA-7858) has increased expression of the xylose isomerase and xylulokinase enzymes that were integrated into the genome as an operon expressed from *Zm*P*gap (*P*gapxylAB* operon). Applicants have further discovered that there is a single new nucleotide change in the promoter of the P*gapxylAB* operon that is responsible for the promoter directing increased expression of operably linked coding regions. The

nucleotide change is new with respect to the sequence of the P*gap* of the P*gapxylAB* operon in strain ZW658 as compared to the sequence of the *Zm*P*gap* of the P*gapxylAB* operon in a precursor strain to ZW658 that did not have increased xylose isomerase and xylulokinase activities. Thus the P*gap* having this single nucleotide change is an improved promoter.

**[0018]** Applicants have in addition discovered that a *Z. mobilis* strain that was separately engineered with the genes encoding the four xylose utilization enzymes and separately adapted for improved xylose utilization (strain 8b, described in U.S. Pat. No. 7,223,575) also has increased expression of the xylose isomerase and xylulokinase enzymes that were integrated into the genome as a P*gapxylAB* operon. Applicants have further discovered that there is a single new nucleotide change in the P*gap* of the P*gapxylAB* operon in the 8b strain that is at a different position than the nucleotide change of the ZW658 P*gap*. Based on the increased expression of the xylose isomerase and xylulokinase enzymes encoded by the P*gapxylAB* operon, the mutant P*gap* of the P*gapxylAB* operon also provides an improved promoter.

**[0019]** The identified new nucleotide changes in the P*gap* of the ZW658 and 8b strain P*gapxylAB* operons are at positions -190 and -89, respectively, with respect to the natural ATG translation initiation codon for glyceraldehyde-3-phosphate dehydrogenase in the CP4 and ZM4 strains of *Z. mobilis*. The discovered nucleotide change at position -190 is from G to T, and at position -89 is from C to T.

**[0020]** The sequence context of the base changes are the important factor, as the position number may change due to sequence variations.

The -190 position is in the sequence context: AACGGTATACT**G**GAATAAATGGTCTTCGTTATGGTATTGATGTTTTT, which is a portion of *Zm*P*gap* of CP4, ZM4, and pZB4 with SEQ ID NOs:1, 2, and 3, respectively,

where the bold and underlined G is the base changed to T by the mutation. This position is -190 in the *Zm*P*gap* sequence of the CP4 and ZM4 strains, but position -189 in pZB4 since in the promoter sequence in pZB4 there is a deletion of T at position -21.

The -89 position is in the sequence context:
CGGCATCACGAA**C**AAGGTGTTGGCCGCGATCGCCGGTAAGTCGGC, which is a portion of *Zm*P*gap* of CP4, ZM4, and pZB4 with SEQ ID NOs:1, 2, and 3, respectively,
where the bold and underlined C is the base changed to T by the mutation. This position is -89 in the *Zm*P*gap* sequence of the CP4 and ZM4 strains, but position -88 in pZB4 since in the promoter sequence in pZB4 there is a deletion of T at position -21. Promoters of the present invention have a nucleotide change in *Zm*P*gap* at position -190, at position -89, or at both of these positions. Preferably the changes are a G to T change at position -190 and a C to T change at position -89. The present promoters comprising these modifications are improved P*gap*s.

**[0021]** Changes to other nucleotides at the -190 and -89 positions may provide improved activity of *Zm*P*gap.* In addition, nucleotide changes at other positions within *Zm*P*gap* may provide improved activity of *Zm*P*gap.*

**[0022]** The naturally occurring sequence of *Zm*P*gap* is not a single sequence, but may have some variation in sequence that has no substantial effect on promoter function. Having no substantial effect on promoter function means that the promoter sequence directs an expression level that is substantially similar to the level of expression directed by a *Zm*P*gap* present in a natural *Zymomonas mobilis* strain. Variation in sequence may naturally occur between different isolates or strains of *Zymomonas mobilis*, such as the difference between the CP4 and ZM4 strains at position -29 with respect to the natural ATG translation initiation codon for glyceraldehyde-3-phosphate dehydrogenase (SEQ ID NOs:1 and 2, respectively), where in CP4 there is an A and in ZM4 there is a G.

**[0023]** In addition to naturally occurring sequence variations, nucleotide changes that do not substantially affect function may occur during routine manipulation procedures including PCR, cloning, transformation, and strain growth as is known to one skilled in the art. An example is the *Zm*P*gap* of pZB4, which has a deletion of T at position -21.

**[0024]** Any nucleotide changes in the *Zm*P*gap* sequence, occurring in different natural or engineered strains, that do not substantially affect promoter function, may be present in the sequence of a *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter such as the deletion of a T after position -21 that is in the *Zm*P*gap* of pZB4 (SEQ ID NO:3). Thus the mutations at positions -190 and -89 described above that do affect promoter function, that is, that substantially improve promoter function, may be made in any of the *Zm*P*gap* sequences with substantially similar activity (natural level) and can co-occurwith variations not affecting function.

**[0025]** Examples of improved P*gap* sequences with the described mutations at positions -190 and/or -89 include the promoter sequence from strain ZW658 (SEQ ID NO:4), from strain 8b (SEQ ID NO:5), and a double mutation of the same *Zm*P*gap* variant which is from pZB4 (SEQ ID NO:6). Additional examples of improved P*gap* sequences are the -190. -89, or double mutation in the *Zm*P*gap* variant from CP4 (SEQ ID NOs:7, 8, and 9, respectively) and the -190. -89, or double mutation in the *Zm*P*gap* variant from ZM4 (SEQ ID NOs:10, 11, and 12, respectively).

**[0026]** In addition, variations in the length of the *Zm*P*gap* occur that do not substantially affect promoter function. The present invention includes improved P*gap*s having the described mutations at position -190 and/or -90 (with respect to the natural ATG translation initiation codon for glyceraldehyde-3-phosphate dehydrogenase in the CP4 and ZM4 strains of *Z. mobilis*) in *Zm*P*gap*s of varying

length that have no substantial change in activity prior to addition of the -190 and/or -89 mutations.

Preparing an improved P*gap*

**[0027]** The described mutations at positions -190 and/or -89 may be introduced into a *Zm*P*gap* nucleic acid molecule by any method known to one skilled in the art. For example, an oligonucleotide having the mutation and surrounding DNA sequence may be synthesized and cloned into a larger promoter DNA fragment, substituting for a segment without the mutation. Primers containing the mutation and some adjacent promoter sequence may be synthesized and used in PCR to prepare the promoter fragment. An entire promoter DNA fragment may be synthesized as multiple oligonucleotides that are ligated together. Site-directed mutagenesis may be used to introduce the mutation(s). In addition, the mutant promoters may be prepared as PCR amplified DNA fragments using DNA from the ZW658 or 8b strain as template.

Expression of xylose isomerase using improved P*gap*

**[0028]** A promoter described herein may be operably linked to a heterologous nucleic molecule that encodes xylose isomerase for directing increased expression of xylose isomerase, as compared to expression from the *Zm*P*gap*. The improved P*gap* and xylose isomerase coding region form a chimeric gene, which also generally includes a 3' termination control region. Termination control regions may be derived from various genes, and are often taken from genes native to a target host cell. The construction of chimeric genes is well known in the art.

**[0029]** Any xylose isomerase coding region may be used in a chimeric gene to express xylose isomerase from an improved P*gap* in the present invention. Xylose isomerase enzymes belong to the group EC5.3.1.5. Examples of suitable xylose isomerase proteins and encoding sequences that may be used are given in Table 1. Particularly suitable examples are those from *E. coli* (SEQ ID NO:42

and 43, respectively), and *Lactobacillus brevis* (SEQ ID NO:44 and 45, respectively).

**[0030]** Many other examples of xylose isomerase proteins and encoding sequences are identified in the literature and in bioinformatics databases well known to the skilled person. Additionally, the encoding sequences described herein or those recited in the art may be used to identify other homologs in nature. For example each of the xylose isomerase encoding nucleic acid fragments described herein may be used to isolate genes encoding homologous proteins from the same or other microbial species. Isolation of homologous genes using sequence-dependent protocols is well known in the art.

**[0031]** Examples of sequence-dependent protocols include, but are not limited to: 1.) methods of nucleic acid hybridization; 2.) methods of DNA and RNA amplification, as exemplified by various uses of nucleic acid amplification technologies [e.g., polymerase chain reaction (PCR), Mullis et al., U.S. Patent 4,683,202; ligase chain reaction (LCR), Tabor, S. et al., *Proc. Acad. Sci.* USA 82:1074 (1985); or strand displacement amplification (SDA), Walker, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 89:392 (1992)]; and 3.) methods of library construction and screening by complementation.

**[0032]** For example, sequences encoding similar proteins or polypeptides to the xylose isomerase coding regions described herein could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired bacteria using methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the disclosed nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis, *supra*). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan (e.g., random primers DNA labeling, nick translation or end-labeling techniques), or RNA probes using available *in vitro* transcription systems. In addition, specific

primers can be designed and used to amplify a part of (or full-length of) the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full-length DNA fragments under conditions of appropriate stringency.

**[0033]** Typically, in PCR-type amplification techniques, the primers have different sequences and are not complementary to each other. Depending on the desired test conditions, the sequences of the primers should be designed to provide for both efficient and faithful replication of the target nucleic acid. Methods of PCR primer design are common and well known in the art (Thein and Wallace, "The use of oligonucleotides as specific hybridization probes in the Diagnosis of Genetic Disorders", in *Human Genetic Diseases: A Practical Approach*, K. E. Davis Ed., (1986) pp 33-50, IRL: Herndon, VA; and Rychlik, W., In Methods in Molecular Biology, White, B. A. Ed., (1993) Vol. 15, pp 31-39, PCR Protocols: Current Methods and Applications. Humania: Totowa, NJ).

**[0034]** Generally two short segments of the described sequences may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the described nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding microbial genes.

**[0035]** Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al., *PNAS USA* 85:8998 (1988)) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be

designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (e.g., BRL, Gaithersburg, MD), specific 3' or 5' cDNA fragments can be isolated (Ohara et al., *PNAS USA* 86:5673 (1989); Loh et al., *Science* 243:217 (1989)).

**[0036]** Alternatively, these xylose isomerase encoding sequences may be employed as hybridization reagents for the identification of homologs. The basic components of a nucleic acid hybridization test include a probe, a sample suspected of containing the gene or gene fragment of interest, and a specific hybridization method. Probes are typically single-stranded nucleic acid sequences that are complementary to the nucleic acid sequences to be detected. Probes are "hybridizable" to the nucleic acid sequence to be detected. The probe length can vary from 5 bases to tens of thousands of bases, and will depend upon the specific test to be done. Typically a probe length of about 15 bases to about 30 bases is suitable. Only part of the probe molecule need be complementary to the nucleic acid sequence to be detected. In addition, the complementarity between the probe and the target sequence need not be perfect. Hybridization does occur between imperfectly complementary molecules with the result that a certain fraction of the bases in the hybridized region are not paired with the proper complementary base.

**[0037]** Hybridization methods are well defined and known in the art. Typically the probe and sample must be mixed under conditions that will permit nucleic acid hybridization. This involves contacting the probe and sample in the presence of an inorganic or organic salt under the proper concentration and temperature conditions. The probe and sample nucleic acids must be in contact for a long enough time that any possible hybridization between the probe and sample nucleic acid may occur. The concentration of probe or target in the mixture will determine the time necessary for hybridization to occur. The higher the probe or target concentration, the shorter the hybridization incubation time needed. Optionally, a chaotropic agent may be added. The chaotropic agent stabilizes

nucleic acids by inhibiting nuclease activity. Furthermore, the chaotropic agent allows sensitive and stringent hybridization of short oligonucleotide probes at room temperature (Van Ness and Chen, *Nucl. Acids Res*. 19:5143-5151 (1991)). Suitable chaotropic agents include guanidinium chloride, guanidinium thiocyanate, sodium thiocyanate, lithium tetrachloroacetate, sodium perchlorate, rubidium tetrachloroacetate, potassium iodide and cesium trifluoroacetate, among others. Typically, the chaotropic agent will be present at a final concentration of about 3 M. If desired, one can add formamide to the hybridization mixture, typically 30-50% (v/v).

**[0038]** Various hybridization solutions can be employed. Typically, these comprise from about 20 to 60% volume, preferably 30%, of a polar organic solvent. A common hybridization solution employs about 30-50% v/v formamide, about 0.15 to 1 M sodium chloride, about 0.05 to 0.1 M buffers (e.g., sodium citrate, Tris-HCl, PIPES or HEPES (pH range about 6-9)), about 0.05 to 0.2% detergent (e.g., sodium dodecylsulfate), or between 0.5-20 mM EDTA, FICOLL (Pharmacia Inc.) (about 300-500 kdal), polyvinylpyrrolidone (about 250-500 kdal) and serum albumin. Also included in the typical hybridization solution will be unlabeled carrier nucleic acids from about 0.1 to 5 mg/mL, fragmented nucleic DNA (e.g., calf thymus or salmon sperm DNA, or yeast RNA), and optionally from about 0.5 to 2% wt/vol glycine. Other additives may also be included, such as volume exclusion agents that include a variety of polar water-soluble or swellable agents (e.g., polyethylene glycol), anionic polymers (e.g., polyacrylate or polymethylacrylate) and anionic saccharidic polymers (e.g., dextran sulfate).

**[0039]** Nucleic acid hybridization is adaptable to a variety of assay formats. One of the most suitable is the sandwich assay format. The sandwich assay is particularly adaptable to hybridization under non-denaturing conditions. A primary component of a sandwich-type assay is a solid support. The solid support has adsorbed to it or covalently coupled to it immobilized nucleic acid probe that is unlabeled and complementary to one portion of the sequence.

Bioinformatic Approaches

**[0040]** Alternatively, because xylose isomerase proteins are so well known and abundant, additional xylose isomerase proteins may be identified on the basis of catalytic site residues and a Profile Hidden Markov Model (HMM) constructed using the Pfam (Pfam: clans, web tools and services: R.D. Finn, J. Mistry, B. Schuster-Böckler, S. Griffiths-Jones, V. Hollich, T. Lassmann, S. Moxon, M. Marshall, A. Khanna, R. Durbin, S.R. Eddy, E.L.L. Sonnhammer and A. Bateman, Nucleic Acids Research (2006) Database Issue 34:D247-D251) identified family of xylose isomerase proteins.

**[0041]** The Profile HMM is prepared using the hmmsearch algorithm of the HMMER software package (Janelia Farm Research Campus, Ashburn, VA). The theory behind Profile HMMs is described in Durbin et al. ((1998) Biological sequence analysis: probabilistic models of proteins and nucleic acids, Cambridge University Press) and Krogh et al. ((1994) J. Mol. Biol. 235:1501–1531), which characterizes a set of proteins based on the probability of each amino acid occurring at each position in the alignment of the proteins of the set.

**[0042]** A Profile HMM for xylose isomerases prepared using 32 xylose isomerase protein sequences with experimentally verified function as referenced in the BRENDA database provides a basis for identification of xylose isomerases. BRENDA is a human-curated database that contains detailed information about enzyme kinetic, physical, and biochemical properties extracted from the experimental literature and with links to the relevant databases (Cologne University BioInformatics Center). The SEQ ID NOs for these 32 proteins are given in Table 1. Using these 32 protein sequences a multiple sequence alignment (MSA) was built using ClustalW with default parameters. The MSA results were used as input data to prepare the Profile HMM that is given in Table 3. In the table, the amino acids are represented by the one letter code. The first line for each position reports the match emission scores: probability for each

amino acid to be in that state (highest score is highlighted). The second line reports the insert emission scores, and the third line reports on state transition scores: M→M, M→I, M→D; I→M, I→I; D→M, D→D; B→M; M→E.

**[0043]** In addition to the Profile HMM, four catalytic site amino acids are characteristic of xylose isomerases: histidine 54, aspartic acid 57, glutamic acid 181, and lysine 183, with the position numbers in reference to the *Streptomyces albus* xylose isomerase sequence. Any protein fitting the xylose isomerase Profile HMM with an Evalue < or = $3x10^{-10}$ and having these four catalytic site residues is a xylose isomerase whose coding region may be constructed in a chimeric gene comprising an improved P*gap* and transformed into a bacterial strain as described herein. Currently 251 proteins in the GenBank sequence database, reduced to a 90% non-redundancy level, match these criteria. These sequences will not all be presented herein with SEQ ID NOs as they can be readily identified by one skilled in the art. Additional sequences fitting these criteria that become available may also be used as described herein.

**[0044]** As known in the art, there may be variations in DNA sequences encoding an amino acid sequence due to the degeneracy of the genetic code. Codons may be optimized for expression of an amino acid sequence in a target host cell to provide for optimal encoded expression.

Engineering bacterial cells for xylose isomerase expression

**[0045]** The chimeric genes described herein are typically constructed in or transferred to a vector for further manipulations. Vectors are well known in the art. Certain vectors are capable of replicating in a broad range of host bacteria and can be transferred by conjugation. The complete and annotated sequence of pRK404 and three related vectors: pRK437, pRK442, and pRK442(H) are available. These derivatives have proven to be valuable tools for genetic manipulation in gram-negative bacteria (Scott et al., *Plasmid* 50(1):74-79 (2003)).

**[0046]** Other well-known vectors may be used in different target host cells. Examples of vectors useful for different hosts are described in co-owned and co-pending U.S. App. Pub. No. US20070092957 A1, pp11-13, which is hereby incorporated herein by reference. Particularly useful for expression in *Zymomonas* are vectors that can replicate in both *E. coli* and *Zymomonas*, such as pZB188 which is described in U.S. Pat. No. 5,514,583. Vectors may include plasmids for autonomous replication in a cell, and plasmids for carrying constructs to be integrated into bacterial genomes. Plasmids for DNA integration may include transposons, regions of nucleic acid sequence homologous to the target bacterial genome, or other sequences supporting integration. An additional type of vector may be a transposome produced using, for example, a system that is commercially available from EPICENTRE®. It is well known how to choose an appropriate vector for the desired target host and the desired function.

**[0047]** Bacterial cells may be engineered by introducing a vector having a chimeric gene comprising an improved P*gap* and operably linked xylose isomerase coding region by well known methods, such as using freeze-thaw transformation, calcium-mediated transformation, electroporation, or conjugation. Any bacterial cell to be engineered for xylose utilization by expressing a xylose isomerase enzyme is a target host cell for transformation to engineer a strain as described herein. Particularly suitable host cells are *Zymomonas* and *Zymobacter.* The introduced chimeric gene may be maintained in the cell on a stably replicating plasmid, or integrated into the genome following introduction.

**[0048]** For engineering a strain with an integrated improved P*gap*-xylose isomerase chimeric gene in the bacterial cell genome, methods may be used that are well known in the art such as homologous recombination, transposon insertion, or transposome insertion. In homologous recombination, DNA sequences flanking a target integration site are placed bounding a spectinomycin-resistance gene or other selectable marker, and the improved

P*gap*-xylose isomerase chimeric gene leading to insertion of the selectable marker and the improved P*gap*-xylose isomerase chimeric gene into the target genomic site. In addition, the selectable marker may be bounded by site-specific recombination sites, so that after expression of the corresponding site-specific recombinase, the resistance gene is excised from the genome. Particularly suitable for integration of the improved P*gap*-xylose isomerase chimeric gene is transposition using EPICENTRE®'s EZ::Tn in vitro transposition system, which is used here in Examples 1 and 6.

Xylose isomerase activity

**[0049]** In the strains described herein, xylose isomerase activity levels are higher than previously described in the art. These strains are engineered to express xylose isomerase at a level to produce at least about 0.1 μmoles product/mg protein/minute, as determined by reacting 20 μL of cell free extract in a reaction mix, at 30°C, comprising 0.256 mM NADH, 50 mM xylose, 10 mM $MgSO_4$, 10 mM triethanolamine, and 1 U/ml sorbitol dehydrogenase, wherein D-xylulose is the product. Strains may express xylose isomerase at a level to produce at least about 0.14, 0.2, or 0.25 μmoles product/mg protein/minute. High expression promoters with the improved P*gap* described herein may be used to express a xylose isomerase coding region to obtain these enzyme activity levels. The high xylose isomerase activity levels in the presence of three additional xylose metabolic pathway enzyme activities described below provides a strain with improved growth on xylose-containing medium.

Engineering of full xylose utilization pathway

**[0050]** In addition to transforming with a chimeric gene comprising an improved P*gap* and xylose isomerase coding region, bacterial strains are also engineered for expression of the three other enzymes needed for xylose utilization: xylulokinase, transaldolase and transketolase, as described in U.S. Pat. No. 5514583, U.S. Pat. No. 5712133, U.S. Pat. No. 6566107, WO 95/28476, Feldmann et al. ((1992) Appl Microbiol Biotechnol 38: 354-361), Zhang et al.

((1995) Science 267:240-243)), and Yanase et al. ((2007) Appl. Environ. Mirobiol. 73:2592-2599).

**[0051]** The presence of genes encoding all four enzymes is known to complete the xylose utilization pathway to produce xylose-utilizing strains. The additional three enzymes may be expressed from individual chimeric genes or from operons including more than one coding region.

**[0052]** Chimeric genes may be constructed by operably linking a promoter, coding region, and a 3' termination control region as described above for a xylose isomerase chimeric gene. The promoter is chosen as one that is active in the target host cell, as well known in the art. Promoters that may be used in *Zymomonas* and *Zymobacter* include *Zm*P*gap* and the promoter of the *Zymomonas* enolase gene. Coding regions for xylulokinase, transaldolase and transketolase may be from any Gram-negative bacterium capable of utilizing xylose, for example *Xanthomonas*, *Klebsiella*, *Escherichia*, *Rhodobacter*, *Flavobacterium*, *Acetobacter*, *Gluconobacter*, *Rhizobium*, *Agrobacterium*, *Salmonella*, and *Pseudomonas*. Examples of protein sequences and their encoding region sequences that may be used are given in Table 2. Preferred are the sequences encoding xylulokinase, transaldolase and transketolase enzymes from *E. coli* (SEQ ID NOs:107, 119, and 131, respectively). These sequences may also be used to identify additional encoding sequences, as described above for xylose isomerase, that may be used to express the complete xylose utilization pathway.

**[0053]** In addition, bioinformatics methods including Pfam protein families and Profile HMMs as described above for xylose isomerase may be applied to identifying xylulokinase, transaldolase and transketolase enzymes. Sequences encoding these enzyme may have diversity due to codon degeneracy and may be codon optimized for expression in a specific host, also as described above.

**[0054]** Operons may be constructed for expression of xylulokinase, transaldolase and transketolase. One or more of the encoding sequences may be operably linked with the xylose isomerase coding region expressed from an improved P*gap*, forming an operon. Typically xylose isomerase and xylulokinase are expressed in one operon, and transaldolase and transketolase are expressed in a second operon, as described in Example 1 herein.

**[0055]** These enzymes may be expressed from chimeric genes or operons located on stably replicating plasmids, or integrated into the genome.

Improved Growth of bacterial strains having improved P*gap*-xylose isomerase chimeric gene

**[0056]** A xylose-utilizing bacterial strain described herein having an improved P*gap*-xylose isomerase chimeric gene, for example a *Zymomonas mobilis* strain, shows improved growth in a medium containing xylose in the absence or presence of other sugars ("mixed sugars"). The mixed sugars include at least one additional sugar to xylose. Any sugar that may provide an energy source for metabolism of the cells, or any sugar that is present in a mixture containing xylose may be included. It is desirable to grow strains of the present invention on sugars that are produced from biomass saccharification. Typically biomass is pretreated, for example as described in Patent Application WO2004/081185 and in co-owned and co-pending US application 60/670437, and then treated with saccharification enzymes as reviewed in Lynd, L. R., et al. (Microbiol. Mol. Biol. Rev. (2002) 66:506-577). Biomass saccharification produces sugars that may typically include a mixture of xylose with glucose, fructose, sucrose, galactose, mannose, and/or arabinose.

**[0057]** For maximal production and efficiency of fermentation it is desirable to grow a strain described herein in medium containing high levels of sugars,

including xylose. This allows the direct use of biomass saccharification sugars, or use with little dilution, thereby reducing fermentation volumes, which is desirable for commercial scale production, such as of ethanol. High sugars concentrations are used so that greater concentrations of product, such as ethanol, may be produced. The mixed sugars concentration in the fermentation medium is typically between about 120 g/L and up to about 300 g/L, more typically between about 150 g/L and about 235 g/L.

**[0058]** In the high concentration mixed sugars conditions desired for commercial production, such as of ethanol, sorbitol may be included in the fermentation medium. Sorbitol (D-sorbitol and/or L-sorbitol) may be present in the medium at concentrations that are between about 2 mM and 200 mM, typically between about 2 mM and 100 mM, or between 5 mM and 20 mM as described in commonly owned and co-pending US Application Publication # 20080286870. Mannitol, galactitol or ribitol may be used in the medium instead of sorbitol, or in combination with sorbitol, as described in commonly owned and co-pending U.S. App. Pub. No. US20080081358.

**[0059]** Under fermentation conditions in xylitol medium, a strain described herein having an improved P*gap*-xylose isomerase chimeric gene has improved growth over a strain with xylose isomerase expressed from a *Zm*P*gap*. The exact improvement will vary depending on the strain background, medium used, and general growth conditions. For example, when grown in media containing 50 g/L xylose, after one hour strains with the improved Pgap-xylose isomerase chimeric gene grew to an OD600 of between about two and five times higher than that of strains without the improved P*gap*, as shown in Example 8, Figure 13A, herein.

Fermentation of improved xylose-utilizing strain

**[0060]** An engineered xylose-utilizing strain with an improved P*gap*-xylose isomerase chimeric gene and genes or operons for expression of xylulokinase, transaldolase and transketolase may be used in fermentation to produce a

product that is a natural product of the strain, or a product that the strain is engineered to produce. For example, *Zymomonas mobilis* and *Zymobacter palmae* are natural ethanolagens. As an example, production of ethanol by a *Z. mobilis* strain of the invention is described.

**[0061]** For production of ethanol, recombinant xylose-utilizing *Z. mobilis* having an improved P*gap*-xylose isomerase chimeric gene is brought in contact with medium that contains mixed sugars including xylose. When the mixed sugars concentration is high such that growth is inhibited, the medium includes sorbitol, mannitol, or a mixture thereof. Galactitol or ribitol may replace or be combined with sorbitol or mannitol. The *Z. mobilis* grows in the medium where fermentation occurs and ethanol is produced. The fermentation is run without supplemented air, oxygen, or other gases (which may include conditions such as anaerobic, microaerobic, or microaerophilic fermentation), for at least about 24 hours, and may be run for 30 or more hours. The timing to reach maximal ethanol production is variable, depending on the fermentation conditions. Typically, if inhibitors are present in the medium, a longer fermentation period is required. The fermentations may be run at temperatures that are between about 30 $^{o}$ C and about 37 $^{o}$ C, at a pH of about 4.5 to about 7.5.

**[0062]** The present *Z. mobilis* may be grown in medium containing mixed sugars including xylose in laboratory scale fermenters, and in scaled up fermentation where commercial quantities of ethanol are produced. Where commercial production of ethanol is desired, a variety of culture methodologies may be applied. For example, large-scale production from the present *Z. mobilis* strains may be produced by both batch and continuous culture methodologies. A classical batch culturing method is a closed system where the composition of the medium is set at the beginning of the culture and not subjected to artificial alterations during the culturing process. Thus, at the beginning of the culturing process the medium is inoculated with the desired organism and growth or metabolic activity is permitted to occur adding nothing to the system. Typically,

however, a "batch" culture is batch with respect to the addition of carbon source and attempts are often made at controlling factors such as pH and oxygen concentration. In batch systems the metabolite and biomass compositions of the system change constantly up to the time the culture is terminated. Within batch cultures cells moderate through a static lag phase to a high growth log phase and finally to a stationary phase where growth rate is diminished or halted. If untreated, cells in the stationary phase will eventually die. Cells in log phase are often responsible for the bulk of production of end product or intermediate in some systems. Stationary or post-exponential phase production can be obtained in other systems.

**[0063]** A variation on the standard batch system is the Fed-Batch system. Fed-Batch culture processes are also suitable for growth of the present *Z. mobilis* strains and comprise a typical batch system with the exception that the substrate is added in increments as the culture progresses. Fed-Batch systems are useful when catabolite repression is apt to inhibit the metabolism of the cells and where it is desirable to have limited amounts of substrate in the medium. Measurement of the actual substrate concentration in Fed-Batch systems is difficult and is therefore estimated on the basis of the changes of measurable factors such as pH and the partial pressure of waste gases such as $CO_2$. Batch and Fed-Batch culturing methods are common and well known in the art and examples may be found in Biotechnology: A Textbook of Industrial Microbiology, Crueger, Crueger, and Brock, Second Edition (1989) Sinauer Associates, Inc., Sunderland, MA, or Deshpande, Mukund V., *Appl. Biochem. Biotechnol.*, 36, 227, (1992), herein incorporated by reference.

**[0064]** Commercial production of ethanol may also be accomplished with a continuous culture. Continuous cultures are open systems where a defined culture medium is added continuously to a bioreactor and an equal amount of conditioned medium is removed simultaneously for processing. Continuous cultures generally maintain the cells at a constant high liquid phase density

where cells are primarily in log phase growth. Alternatively, continuous culture may be practiced with immobilized cells where carbon and nutrients are continuously added, and valuable products, by-products or waste products are continuously removed from the cell mass. Cell immobilization may be performed using a wide range of solid supports composed of natural and/or synthetic materials as is known to one skilled in the art.

**[0065]** Continuous or semi-continuous culture allows for the modulation of one factor or any number of factors that affect cell growth or end product concentration. For example, one method will maintain a limiting nutrient such as the carbon source or nitrogen level at a fixed rate and allow all other parameters to moderate. In other systems a number of factors affecting growth can be altered continuously while the cell concentration, measured by medium turbidity, is kept constant. Continuous systems strive to maintain steady state growth conditions and thus the cell loss due to medium being drawn off must be balanced against the cell growth rate in the culture. Methods of modulating nutrients and growth factors for continuous culture processes as well as techniques for maximizing the rate of product formation are well known in the art of industrial microbiology and a variety of methods are detailed by Brock, *supra*.

**[0066]** Particularly suitable for ethanol production is a fermentation regime as follows. The desired *Z. mobilis* strain of the present invention is grown in shake flasks in semi-complex medium at about 30 $^{o}$C to about 37 $^{o}$C with shaking at about 150 rpm in orbital shakers and then transferred to a 10 L seed fermentor containing similar medium. The seed culture is grown in the seed fermentor anaerobically until $OD_{600}$ is between 3 and 6, when it is transferred to the production fermentor where the fermentation parameters are optimized for ethanol production. Typical inoculum volumes transferred from the seed tank to the production tank range from about 2% to about 20% v/v. Typical fermentation medium contains minimal medium components such as potassium phosphate (1.0 – 10.0 g/l), ammonium sulfate (0- 2.0 g/l), magnesium sulfate (0 – 5.0 g/l), a

complex nitrogen source such as yeast extract or soy based products (0 – 10 g/l). A final concentration of about 5 mM sorbitol or mannitol is present in the medium. Mixed sugars including xylose and at least one additional sugar such as glucose (or sucrose), providing a carbon source, are continually added to the fermentation vessel on depletion of the initial batched carbon source (50-200 g/l) to maximize ethanol rate and titer. Carbon source feed rates are adjusted dynamically to ensure that the culture is not accumulating glucose in excess, which could lead to build up of toxic byproducts such as acetic acid. In order to maximize yield of ethanol produced from substrate utilized, biomass growth is restricted by the amount of phosphate that is either batched initially or that is fed during the course of the fermentation. The fermentation is controlled at pH 5.0 – 6.0 using caustic solution (such as ammonium hydroxide, potassium hydroxide, or sodium hydroxide) and either sulfuric or phosphoric acid. The temperature of the fermentor is controlled at 30 $^{o}$C - 35 $^{o}$C. In order to minimize foaming, antifoam agents (any class- silicone based, organic based etc) are added to the vessel as needed. An antibiotic, for which there is an antibiotic resistant marker in the strain, such as kanamycin, may be used optionally to minimize contamination.

**[0067]** Any set of conditions described above, and additionally variations in these conditions that are well known in the art, are suitable conditions for production of ethanol by a xylose-utilizing recombinant *Zymomonas* strain.

## EXAMPLES

**[0068]** The Examples illustrate the inventions described herein.

## GENERAL METHODS

**[0069]** Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, 2$^{nd}$ ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989)

(hereinafter "Maniatis"); and by Silhavy, T. J., Bennan, M. L. and Enquist, L. W., *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1984); and by Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, published by Greene Publishing Assoc. and Wiley-Interscience, Hoboken, NJ (1987).

**[0070]** The meaning of abbreviations is as follows: "kb" means kilobase(s), "bp" means base pairs, "nt" means nucleotide(s), "hr" means hour(s), "min" means minute(s), "sec" means second(s), "d" means day(s), "L" means liter(s), "ml" means milliliter(s), "μL" means microliter(s), "μg" means microgram(s), "ng" means nanogram(s), "mM" means millimolar, "μM" means micromolar, "nm" means nanometer(s), "μmol" means micromole(s), "pmol" means picomole(s), "Cm" means chloramphenicol, "$Cm^r$ " means chloramphenicol resistant, "$Cm^s$" means chloramphenicol sensitive, "$Sp^r$ " means spectinomycin resistance, "$Sp^s$" means spectinomycin sensitive, "XI" is xylose isomerase, "XK" is xylulokinase, "TAL" is transaldolase, "TKT" is transketolase, "EFT" means elapsed fermentation time, "RM" means rich medium containing 10 g/L yeast extract plus 2 g/L $KH_2PO_4$, "MM" means mating medium containing 10 g/L yeast extract, 5 g/L tryptone, 2.5 g/L $(NH_4)_2SO_4$ and 0.2 g/L $KH_2PO_4$.

Preparation of Cell-Free Extracts of *Zymomonas* for Enzymatic Assays

**[0071]** Cells were grown in 50 ml of RM + 2% glucose at 30°C overnight to an $OD_{600}$ of 1.0-1.2. Cells were harvested by centrifugation at 4500 rpm for 10 min at 4°C. The supernatant was discarded and the cell pellet washed with 25 ml ice-cold sonication buffer (10 mM Tris, pH 7.6, 10 mM $MgCl_2$), followed by centrifugation at 4500 rpm for 10 min. The pellet was resuspended in 2.0-2.5 ml sonication buffer plus 1 mM dithiothreitol. A 500 μL aliquot was centrifuged for 1 min in an eppendorf centrifuge at 4°C. Most of supernatant was discarded, leaving about10-20 μL behind to keep the pellet from drying out. The cells were frozen and stored at about 80 °C until assayed. Prior to assay, the cells were thawed and resuspended with 500 μL of sonication buffer plus 1 mM

dithiothreitol. The mix was sonicated 2x for 45 seconds at 62% duty cycle and an output control of 2 using a Branson sonifier 450, letting samples cool about 3-5 min between sonications. Samples were centrifuged at14,000 rpm for 60 min in a Beckman microfuge at 4°C. The supernatant was transferred to a new tube and kept at 4°C. The Pierce BCA assay was used for determining protein concentrations.

**[0072]** The transketolase (TKT) assay was usually performed first since this enzyme is more labile than the others. A diagram of the TKT assay is shown in Figure 1A.

**[0073]** In a microplate assay, 20 μL of cell free extract was added to each well in a reaction mix, at 30°C, that included the following final concentrations of components: 0.37 mM NADP, 50 mM TrisHCl pH 7.5, 8.4 mM Mg $Cl_2$, 0.1 mM TPP ((thiamine pyrophosphate chloride), 0.6 mM E4P (erythrose-4-phosphate), 4mM BHP (betahydroxypyruvate), 4U/ml PGI (phosphoglucose isomerase), and 4 U/ml G6PD (glucose-6-phosphate dehydrogenase). The $A_{340}$ was read on a plate reader for 3-5 min. TKT activity was calculated as follows:

1 unit corresponds to the formation of 1 μmol of D-fructose 6-phosphate / min at 30°C.

U (μmole/min) = slope ($dA_{340}$/min) * volume of reaction (μL) / 6220 / 0.55 cm

(moles of NADP→NADPH is 6220 $A_{340}$ per mole per L in a 1 cm cuvette)

(pathlength of 200 μL per well in microplate=0.55 cm)

Specific Activity (μmole/min-mg) = μmole/min / protein concentration (mg)

**[0074]** The basis of the transaldolase (TAL) assay is shown in Figure 1B. In a microplate assay, 20 μL of cell free extract was added to each well in a reaction mix, at 30°C, that included the following final concentrations of components: 0.38 mM NADH, 87 mM triethanolamine, 17 mM EDTA, 33 mM F6P (fructose-6-phosphate), 1.2 mM E4P (erythrose-4-phosphate ), 2.0 U/ml GDH (Glycerol-3-phosphate dehydrogenase), and 20 U/ml TPI (Triose phosphate isomerase ).

The plate was incubated for 5 min., then the $A_{340}$ was read for 3-5 min. TAL activity was calculated as follows:

1 unit corresponds to the formation of 1 μmol of D-glyceraldehyde per minute at 30°C

U (μmole/min) = slope ($dA_{340}$/min) * volume of reaction (μL) / 6220 / 0.55 cm

(moles of NADH→NAD is 6220 $A_{340}$ per mole per L in a 1 cm cuvette)

(pathlength of 200 μL per well in microplate=0.55 cm)

Specific Activity (μmole/min-mg) = μmole/min / protein

**[0075]** The basis of the xylose isomerase (XI) assay is shown in Figure 1C. In a microplate assay, 20 μL of cell free extract was added to each well in a reaction mix, at 30°C, that included the following final concentrations of components: 0.256 mM NADH, 50 mM xylose, 10 mM $MgSO_4$, 10 mM triethanolamine, and 1U/ml SDH (sorbitol dehydrogenase). The $A_{340}$ was read on a plate reader for 3-5 min. XI activity was calculated as follows:

1 unit of XI corresponds to the formation of 1 μmole of D-xylulose per minute at 30°C

U (μmole/min) = slope ($dA_{340}$/min) * volume of reaction (μL) / 6220 / 0.55 cm

(moles of NADHP→NAD is 6220 $A_{340}$ per mole per L in a 1 cm cuvette)

(pathlength of 200 μL per well in microplate=0.55 cm)

Specific Activity (μmole/min-mg) = μmole/min / protein concentration (mg)

**[0076]** The basis of the xylulokinase (XK) assay is shown in Figure 1D. In a microplate assay, 20 μL of cell free extract was added to each well in a reaction mix, at 30°C, that included the following final concentrations of components:0.2 mM NADH, 50 mM Tris HCl pH 7.5, 2.0 mm $MgCl_2$-$6H_2O$, 2.0 M ATP 0.2 M PEP (phosphoenolpyruvate), 8.5 mM D-xylulose, 5 U/ml PK (pyruvate kinase), and 5 U/ml LDH (lactate dehydrognase). The $A_{340}$ was read on a plate reader for 3-5 min. XI activity was calculated as follows:

1 unit corresponds to the formation of 1 μmole of D-xylulose to D-xylulose-5-phosphate per minute at 30°C

U (μmole/min) = slope ($dA_{340}$/min) * volume of reaction (μL) / 6220 / 0.55 cm

(moles of NADH→NAD is 6220 $A_{340}$ per mole per L in a 1 cm cuvette)

(pathlength of 200 μL per well in microplate=0.55 cm)

Specific Activity (μmole/min-mg) = μmole/min / protein concentration (mg)

HPLC Method

**[0077]** The analysis was done with an Agilent 1100 series HPLC and Agilent ChemStation software for LC 3D. The column was BioRad Aminex HPX-87H (HPLC Organic Analysis Column 125-0140) with BioRad Micro-Guard Cartridge Cation-H (125-0129). The operating conditions were:

| | |
|---|---|
| Flow | 0.6 ml/min |
| Solvent | 0.01N $H_2SO_4$ |
| Stop Time | 25 min |
| Injection Volume | 5 μL |
| Auto Sampler | Temp Control @ 10°C or 4 °C |
| Column Temp | 55°C |
| Detector | Refractive Index (40°C) with External Standard Calibration Curves |

## EXAMPLE 1

### CONSTRUCTION OF XYLOSE-FERMENTING *ZYMOMONAS MOBILIS* STRAINS

**[0078]** As described in commonly owned and co-pending U.S. App. Pub. No. US20080286870, strains of xylose-fermenting *Zymomonas mo*bilis were constructed by integrating two operons, P*gapxylAB* and P*gaptaltkt*, containing four xylose-utilizing genes encoding xylose isomerase, xylulokinase, transaldolase and transketolase, into the genome of ZW1 (ATCC #31821) via sequential transposition events, followed by adaptation on selective media containing xylose. Previously, a xylose-fermenting *Zymomonas mobilis* strain called 8b was constructed, as described in U.S. App. Pub. No. 20030162271, by integrating the two operons P*gapxylAxylB* and P*enotaltkt,* along with selectable antibiotic markers, into the genome of *Zymomonas mob*ilis 5C via a combination of homologous recombination and transposon approaches followed by

adaptation and NTG mutagenesis. In the preparation of new strains, transposition (Epicentre's EZ::Tn in vitro transposition system) was used, as opposed to site specific homologous recombination, because this approach offers the advantages of multiple choices of integration sites and relatively high insertion frequency. The four genes encoding the xylose utilization enzymes were arranged and cloned as two separate operons: P*gapxylAB* and P*gaptaltkt* for the integration. An antibiotic resistance marker, a chloramphenicol resistance ($Cm^r$) gene flanked by two P1 phage Cre-recombinase recognition sequences (*loxP*), was attached to each operon for the selection of integrants. The integration of the two operons was accomplished in a two-step, sequential manner: P*gaptaltkt* followed by P*gapxylAB*. Cm resistance selection was used in both integration events, since it was removed by expressing a Cre recombinase on a plasmid followed by curing of the plasmid after each integration. This process allowed the use of the same antibiotic marker for selection multiple times. More importantly, it allowed the removal of the antibiotic marker introduced for selection of the integration of the operons. This process eliminated the negative impact of antibiotic resistance gene(s) on the fermentation strain for commercial use.

Construction of pMODP*gaptaltkt*Cm for Transposition

**[0079]** As described in U.S. App. Pub. No. 20030162271 (Example 9 therein), a 2.2 kb DNA fragment containing the transketolase (tkt) coding region from *E. coli* was isolated from pUCtaltkt (U.S. App. Pub. No. 20030162271) by BglII/XbaI digestion and cloned in a pMOD (Epicentre Biotechnologies, Madison, WI) vector digested with BamHI/XbaI, resulting in pMODtkt. A PCR fragment named P*gaptal* was generated by fusing the promoter region of the *Zymomonas mobilis gap* (P*gap*; glyceraldehyde-3-phosphate dehydrogenase) gene to the coding region of *E. coli* transaldolase (tal) as follows. A P*gap* fragment was amplified from pZB4, the construction of which is described in U.S. Pat. No. 5514583 (Example 3), using primers with SEQ ID NOs:13 and 14. pZB4 contains a P*gap*-xylA/xylB operon and a P*eno*-tal/tkt operon. A *tal* coding region fragment was

amplified from pZB4 using primers with SEQ ID NOs: 15 and 16. A $P_{gap}$*tal* fragment was amplified using the P*gap* and *tal* fragments as template using primers with SEQ ID NOs:17 and 18. This fragment was digested with XbaI and cloned into the plasmid pMOD*tkt,* upstream of the tkt coding region. A *loxP*::Cm fragment was generated by PCR using Cmlox(F,sfi) and Cmlox(R,sfi) primers (SEQ ID NOs:19 and 20) and pZB186 as the template. pZB186 is a combination of a native *Z. mobilis* plasmid and pACYC184, described in US514583 (Example 3) and Zhang et al. ((1995) Science 267:240-243). Finally, the *loxP*::Cm PCR fragment was inserted in the SfiI site of the plasmid containing P*gaptaltkt* to form the integrative plasmid pMODP*gaptaltkt*Cm. In this plasmid, the P*gaptaltkt* *loxP*::Cm fragment was inserted between two mosaic ends (transposase binding sites) in the pMOD vector. The complete nucletotide sequence for the pMODP*gaptaltkt*Cm plasmid is given as SEQ ID NO:21.

Transposition and transformation of pMODP*gaptaltkt*Cm in ZW1

**[0080]** Plasmid pMOD is a pUC-based vector, and therefore is a non-replicative vector in *Zymomonas*. Plasmid pMODP*gaptaltkt*Cm was treated with transposase in the presence of $Mg^{2+}$ at room temperature for one hour and used to transform ZW1 cells by electroporation (using a BioRad Gene Pulser set at 200 ohms, 25 μF and 16 kV/cm). Electroporated cells were incubated in a mating medium (MM), which consists of 10 g/L yeast extract, 5 g/L tryptone, 2.5 g/L $(NH_4)_2SO_4$, 0.2 g/L $K_2HPO_4$ ) supplemented with 50 g/L glucose and 1 mM $MgSO_4$ for 6 hours at 30° C. The transformation mixture was plated on agar plates containing 15 g/L Bacto agar in MM supplemented with 50 g/L glucose and 120 μg/mL chloramphenicol and incubated anerobically at 30 °C. The transformants were visible after about 2 days. The transformation/transposition frequency was approx. $3x10^1$/μg DNA.

**[0081]** A total of 39 $Cm^r$ transformant colonies was obtained. Twenty-one colonies were picked and further analyzed by PCR and enzymatic activity assays. PCR using primers SEQ ID NOs:22 and 23 confirmed the presence of a 3 kb DNA fragment containing *tal* and *tkt* coding regions in the transformants. Back transformation with plasmid DNA from the 21 integrant colonies generated no back transformants in *E. coli* suggesting the *tal* and *tkt* were integrated in the genome of ZW1. These integrants were tested for transaldolase and transketolase activities using protocols modified for microplates (General Methods). The Pierce BCA protein assay was used for the determination of protein concentrations. The transformants were grown up in RM medium containing 2% (w/v) glucose supplemented with 120 μg/ml chloramphenicol) in 50 ml conical centrifuge tubes at 30°C. The control strains 8b and ZW1 were grown up as well (RM plus 2% glucose was used for ZW1) for enzymatic assays. Cells were harvested when the $OD_{600}$ reached 1.0. Cells were washed once and resuspended in sonication buffer (10 mM Tris-HCl, pH 7.6 and 10 mM $MgCl_2$). Enzymatic assays were conducted as described in U.S. App. Pub. No. 20030162271. Units are given as μmole/min-mg. All samples had transaldolase and transketolase activities except for one.

**[0082]** Southern hybridization was performed on genomic and plasmid DNA of selected integrants digested with PstI using a *tkt* probe. ZW1 DNA did not hybridize with the *tkt* probe. A common 1.5 kb band was visible in all integrant genomic DNA samples, which is the expected DNA fragment between a PstI site in *tkt* and a PstI site in *tal*. A second visible high molecular weight (6 kb or greater) band was unique between independent lines T2, T3, T4 and T5 indicating a separate genomic integration site in each line. Interestingly, both plasmid and genomic DNA of T5 hybridized with the *tkt* probe indicating it was likely that P*gaptaltkt* was also integrated in T5 on the native plasmid. These four strains (T2, T3, T4 and T5) were selected for further Cre treatment to remove the $Cm^r$ marker.

Cre treatment to remove $Cm^r$ marker from *taltkt* integrants

**[0083]** To remove the $Cm^r$ marker from the chromosome, T2, T3, T4 and T5 were transformed with pZB188/Spec-Cre. This plasmid is a derivative of the *Zymomonas-E.coli* shuttle vector pZB188 [Zhang et al. (1995) Science 267:240-243; US 5514583] that contains an expression cassette for Cre Recombinase. pZB188/Spec-Cre is identical to the Cre Expression vector that is described In Example 10 (pZB188/Kan-Cre), except that it has a spectinomycin-resistance gene instead of a kanamycin-resistance gene. The transformants were selected on MM agar plates supplemented with 2% glucose and 200 μg/ml spectinomycin). $Sp^r$ resistant colonies were picked onto RM agar plates supplemented with 2% glucose and 200 μg/ml spectinomycin and RM agar plates supplemented with 2% glucose and 120 μg/mL Cm. One hundred percent of the colonies picked were $Cm^s$ indicating the high efficiency excision of $Cm^r$ by Cre. $Sp^rCm^s$ transformants were cultured in RM plus 2% glucose at 37°C for 2 to 5 daily transfers to cure pZB188aadACreF. At each transfer, cells were diluted and plated on RM plus 2% glucose agar plates for picking onto additional plates of the same medium with or without 200 μg/mL Sp. $Sp^s$ colonies were analyzed by PCR to confirm the loss of pZB188aadACreF. The plasmid-cured descendents of the integrants were named T2C, T3C, T4C and T5C. To examine whether these transposition integrants were stable, these 4 strains were grown in RM plus 2% glucose and then transferred to 10 ml of the same medium and grown at 37°C in duplicate test tubes. Cells were transferred daily for ten days, or approximately 100 generations. Colonies were diluted and plated onto RMG plates for colony isolation after the 1st and 10th transfers. Twelve colonies from each transfer of each strain tested positive for the presence of P*gaptaltkt* by colony PCR using 5' P*gap* and 3' *tkt* primers (SEQ ID NOs; 13 and 23). Transaldolase and transketolase activities were also measured for isolates after the 1st and 10th transfers (as described in General Methods). All 4 integrants had similar levels of both TAL and TKT activities after 100 generations on the non-selective medium, suggesting that these integrants were genetically stable.

Construction of pMODP*gapxylAB*Cm for Transposition

**[0084]** The next step was to further integrate the P*gapxylAB loxP*::Cm operon into the ZW1::P*gaptaltkt* integrants (T2C, T3C, T4C and T5C). The integrative plasmid pMODP*gapxylAB*Cm was constructed based on the plasmid pMODP*gaptaltkt*Cm (described above). The P*gaptaltkt* DNA fragment was removed by SacI/SfiI digestion. An adaptor fragment containing SacI, NotI, and SfiI restriction sites was introduced by ligation. A NotI fragment of P*gapxylAB*, that was isolated from pZB4 (US 5514583), was then cloned in the NotI site of the adaptor. Xylose isomerase (XI) is encoded by *xylA* and xylulokinase (XK) is encoded by *xylB*. The complete nucletotide sequence for the pMODP*gapxylAB*Cm plasmid is given as SEQ ID NO:24.

Transposition and transformation of pMODP*gapxylAB*Cm in T2C, T3C, T4C and T5C

**[0085]** Using a similar approach to the integration of P*gaptaltkt*Cm, T2C, T3C, T4C and T5C were transformed/transposed with pMODP*gapxylAB*Cm (described above) treated with transposase. Six integrants (T3CCmX1, T3CCmX2, T3CCmX3, T4CCmX1, T5CCmX1, T5CCmX2) were obtained in 2 transformation/transposition experiments following Cm selection. All were confirmed for the presence of *xylAB* by PCR using two sets of primers: SEQ ID NOs:25, and 26, and SEQ ID NOs:15 and 16 except for T2CcmX1 and T2CcmX6 from which no PCR fragment was detected using the primers SEQ ID NOs:25 and 26.

**[0086]** The integrants, including the 2 PCR negative lines, were assayed for XI, XK, TAL and TKT activities (General Methods). The results shown in Figures 2 and 3 indicated that the six *xylAB* integrants T3CCmX1, T3CCmX2, T3CCmX3, T4CCmX1, T5CCmX1, and T5CCmX2 all had XI, XK, TAL and TKT activities. XI and XK activities were newly acquired as compared to the negative parental controls (Figure 2). TAL and TKT activities were maintained as in the parental

controls. All results indicated that the proteins were made and functional. Enzyme activity levels varied, with TI and XK activities similar to those of ZW1 integrants transformed/transposed with the same plasmid. The levels of activities of XI, XK, TAL and TKT were lower than those in strain 8b.

**[0087]** The integration of the *xylAB* operon was confirmed by Southern hybridization. Both genomic and plasmid DNA of the 6 lines were digested with SphI and hybridized to a digoxenin labeled *xylB* probe. A common band of about 3 kb, which is generated from an SphI site in xylB and another SphI site in the adjacent cloning sites on the pMOD vector, was present in all genomic DNA samples, and in addition, higher molecular weight hybridizing bands in the genomic DNA samples indicated that there were four sites of integration for the P*gapxylAB* operon in the chromosome. T3CCmX1 and T3CCmX2 appear to have the same integration site, T3CCmX3 and T4CCmX1 may have the same integration site, and T5CCmX1 and T5CCmX2 each have a separate integration site. Digestion of the same DNA with PstI followed by Southern hybridization with the *tkt* probe demonstrated that each integrant had the same hybridization pattern as its respective parental strain.

Adaptation of the ZW1::P*gaptaltkt* P*gapxylAB* Cm integrants on xylose media

**[0088]** Despite the presence of all four enzymatic activities for xylose utilization, previous observations (U.S. App. Pub. No. 20030162271) indicated that the integrants may not grow on xylose immediately. Growth on xylose may occur after prolonged incubation on xylose medium (either in test tubes or on plates), a process called adaptation.

**[0089]** The strains were adapted as follows. ZW1::P*gaptaltkt*P*gapxylAB*Cm integrant strains were inoculated into test tubes containing RMX (containing 10 g/l yeast extract, 2 g/l $KH_2PO_4$, 20 g/l or 2% (w/v) xylose as well as onto MMGX or MMX plates (10 g/L yeast extract, 5 g/L of tryptone, 2.5 g/L of $(NH_4)_2SO_4$, 0.2 g/L $K_2HPO_4$, 1 mM $MgS0_4$, 1.5% (w/v) agar, 0.025% (w/v) glucose and 4% (w/v)

xylose or just 4% (w/v) xylose). The low level of glucose was used to support initial growth to increase the chance of mutation during adaptation. One of at least five attempts at adaptation on xylose in both cultures and plates was successful. After 10 days of anaerobic incubation at 30°C, 17 and 19 colonies were visible on MMGX plated with T3CCmX1 and T3CCmX2 cells, respectively. The colonies were small and looked unhealthy (transparent) on the plates. Twelve colonies (four from T3CCmX1 plating: T3CCmX11, T3CCmX12, T3CCmX13 and T3CCmX110; eight from T3CCmX2 plating: T3CCmX24, T3CCmX25, T3CCmX26, T3CCmX27, T3CCmX28, T3CCmX29, T3CCmX211 and T3CCmX212) were inoculated in RMGCm120 and transferred into 3 ml RMX for further adaptation to obtain lines that were able to grow faster on xylose.

**[0090]** Adaptation of integrants in test tubes containing 3 ml RMX was conducted at 30°C. $OD_{600}$ was constantly monitored in a Spectronic 601 spectrophotometer. When the growth reached mid-log phase, the cultures were transferred into fresh tubes of RMX. This process was continued for 7 transfers. The growth rates and final ODs (non-linear readings) were improved over the transfers.

**[0091]** At the 6$^{tth}$ transfer, the cultures were streaked out on RMX plates to isolate single colonies. Three integrants grew faster than others on RMX streaked plates: T3CCmX13, T3CCmX26 and T3CCmX27, which are referred to as X13, X26 and X27 in the tables and discussion below. To screen for the best xylose growers, four large (L1-4) and four small (S1-4) colonies each for TX13, X26 and X27 were selected and grown in RMX test tubes so that growth, sugar utilization, and ethanol production could be monitored. Colonies were grown overnight at 30°C followed by inoculation of $OD_{600}$=0.05 into 3 ml of RMX in test tubes in duplicates. X27 grew more slowly in RMG than the other cultures and was inoculated again 6.5 hrs later. After 69 hrs (62.5 hrs for X27), samples were taken for HPLC analysis (General Methods). Figure 4 charts the average ethanol yield (% of theoretical yield) and xylose utilization (%) for cultures at 69 hours

(62.5 hr for all X27 cultures). There was no significant difference between the large and small colonies. Although the performance of X27 was better as compared to X26 on xylose, it showed slower growth on glucose. Therefore, the top performers, large colonies of X13 (X13L3) and X26 (X26L1), were chosen for further evaluation in pH-controlled fermentations. The fermentations were conducted in RMG(6% glucose), RMX(6% xylose) and RMGX(8%:4%; glucose:xylose) at 37°C for strains X13L3 and X26L1, as well as the control strain 8b. Fermentation of glucose by X13L3 and X26L1 grown in RMG(6%) and RMGX(8%:4%) proceeded rather quickly. The fermentation of xylose in the RMGX(8%:4%) was slower for both X13L3 and X26L1 as compared to that of strain 8b. In addition, growth on RMX(6%) at 37°C occurred after a long lag for both X13L3 and X26L1. Several isolates, X13b, X13c and X13FL, were recovered from RMX(6%) fermentations. These isolates along with the original strains X13a (an isolate of X13L3) and X26 were subjected to Cre treatment ,as described previously in this Example, to remove the $Cm^r$ marker from ZW1::P*gaptaltkt*P*gapxylAB*Cm strains. The resulting Cre treated, $Cm^r$-free integrants were named: X13aC, X13bC, X13cC, X13FLC and X26C.

## EXAMPLE 2

### ADAPTATION AND SELECTION OF STRAIN ZW658

**[0092]** As described earlier, adaptation of the initial ZW1::P*gap*taltktP*gap*xylABCm strains on RMX at 30 °C greatly improved the growth of strains in these conditions. However, the adapted strains suffered a long lag during growth and fermentation in RMX(6%) at 37 °C. To further improve the integrants for xylose fermentation at preferred process conditions including higher sugar concentration and temperature, the evolutionary or adaptation process was continued in RMX(5%) at 37 °C. Serial transfers were conducted and the best growers were selected. Integrants used in this process included X13aC, X13bC, X13cC, X26C and X13FLC. These 5 strains were grown in RMX at 30°C for 6 transfers before being transferred to RMX(5%) at 37°C for another 5 to 16 transfers. During and after all the transfers cultures were streaked on RMX

plates and incubated at 37°C to isolate single colonies. Large colonies were further streaked on RMX plates and incubated at 37°C for 3 to 4 times to purify the colonies. Final large colonies were selected for growth testing in RMX(5%) at 37°C.

Evaluation of strains from adaptation in RMX(5%) medium at 37°C

**[0093]** Eighteen colonies isolated after adaptation with serial transfers were tested in RMX(5%) test tubes at 37°C initially. Twelve strains were selected for a 2nd test tube evaluation. Strain 8b was included in all the evaluations for comparison. The 18 colonies were grown up in RMG at 37°C overnight, centrifuged and the cells were inoculated into 4 ml of RMX(5%) at 37°C, statically in test tubes for the 1st evaluation. Based on the growth ($OD_{600}$, non-linear) and end point HPLC results (low residual xylose and high ethanol), 12 strains were selected for the 2nd evaluation.

**[0094]** One of the purposes of the 2nd evaluation was to test the stability of improved growth on xylose and xylose utilization capability of the strains. All 12 strains were subjected to a stability study to see whether the adapted strains were stable after being exposed to a non-selective medium in which they were serially transferred in at 37°C for 50 generations. Cultures before and after RMG(5%) transfers were inoculated in RMX(5%) test tubes and grown at 37°C for evaluation. The non-linear ODs were monitored by direct reading of test tubes in a Spectronic 601 spectrophotometer. The ODs at the 70th hour of growth in RMX(5%) before and after 50 generations of growth in RMG are plotted in Figure 5. The results indicated that most strains were stable after 50 generations in RMG at 37°C. The endpoint (at stationary phase) supernatants were also analyzed by HPLC for xylose and ethanol concentrations. The low residual xylose and high ethanol concentrations in these cultures supported the fact that the strain grew and fermented xylose well.

**[0095]** Based on the results from the above test tube evaluation (low residual xylose, high ethanol concentration and higher OD) and a subsequent microtiter plate growth screening with high concentrations of glucose and/or xylose (up to 20%) and mixtures of glucose and xylose with acetate to select better growers in high sugars and in the presence of acetate, such as strain #26, designated as ZW658, which exhibited the best overall performance

## EXAMPLE 3

### ASSAY OF PENTOSE PHOSPHATE PATHWAY ENZYME ACTIVITIES

**[0096]** The activities of the four xylose utilization enzymes encoded by integrated genes (described in Example 1)were measured as described in the General Methods for three of the strains selected for adaptation at high sugar and 37$^0$C (of Example 1) and were compared to activities of the same enzymes in the further adapted strain ZW658 (of Example 2). The results, expressed as μmoles product/mg protein/minute are shown in Table 4.

Table 4. Enzyme activities in different xylose-utilizing adapted *Z. mobilis* strains

| Strain | Xylose isomerase | Xylulokinase | Transaldolase | Transketolase |
|---|---|---|---|---|
| X13bC | 0.033 +/-0.013 | 1.15 +/-0.13 | 1.66 +/-0.5 | 0.22 +/-0.02 |
| ZW658 | 0.25 +/-0.033 | 4.41 +/-0.21 | 2.67 +/-1.0 | 0.19 +/-0.05 |

**[0097]** The activity levels for both members of the *xylAB* operon were increased by about 4 to 8 fold in the further adapted strain ZW658 as compared to levels in the partially adapted precursor strains. There was little or no change in the expression level of enzymes from the tal/tkt operon between ZW658 and the partially adapted precursor strains.

## EXAMPLE 4

### SEQUENCE COMPARISON OF THE PROMOTER REGIONS OF THE XYLAB OPERONS IN A PARTIALLY ADAPTED STRAIN AND IN ZW658

**[0098]** Since a clear change in the enzyme activity levels of the products of both genes under the control of the GAP promoter (P*gap*) driving xylAB was a noted outcome of the adaptation that led to ZW658, the promoter region of that operon from a partiallyadapted strain (of Example 1; subsequently given the strain number ZW641) and from ZW658 were amplified by PCR and sequenced. A PCR fragment was prepared using a forward PCR primer (PC11; SEQ ID NO:27) from the recG coding region where the P*gapxylAB* operon was integrated and a reverse primer from the xylA coding region (PC12; SEQ ID NO:28). The resulting 961 bp PCR product was sequenced using primers LM121, LM122, and LM123 (SEQ ID NOs:29, 30, and 31). The promoter sequence from ZW641 is given in SEQ ID NO:3 and that from ZW658 in SEQ ID NO:4. These promoter sequences were both found to differ at one position from the published sequence of the P*gap* in the Z. mobilis strain CP4 (SEQ ID NO:1): a 1 base deletion (of a T) after position -21, counting towards the 5' end starting upstream of the ATG start codon for the GAP coding region. This sequence change does not contribute to any difference in expression between the *Pgap* of ZW641 and *Pgap* of ZW658 since it is present in both strains. In addition to this common change- there was also a single base pair difference between the ZW641 and ZW658 *Pgap* sequences. The G at position -189 with respect to the coding region start ATG for XylA in the sequence from the ZW641 strain was replaced by a T in the sequence from ZW658. No other changes between the two sequences were noted and it seemed possible that a change in expression level due to this single base change in the GAP promoter region might be responsible for the increased enzyme activities found for both proteins encoded by genes under the control of that promoter.

## EXAMPLE 5

## CONSTRUCTION OF A XYLOSE ISOMERASE EXPRESSION VECTOR *FOR Z. MOBILIS* THAT HAS THE SAME P*GAP* THAT DRIVES THE XYLA/B OPERON IN *Z. MOBILIS* ZW641

**[0099]** A plasmid construct that confers resistance to spectinomycin and expression of *E. coli* xylose isomerase in *Z. mobilis* (pZB188/aada-GapXylA;

where Gap represents the promoter) was generated as described below using an *E. coli/Z. mobilis* shuttle vector (pZB188) as starting material (Figure 6A). Steps involved in the construction of pZB188 are disclosed in US 5,514,583. Briefly, this 7008 bp plasmid is able to replicate in *E. coli* and *Z. mobilis* because it has two different origins of replication, one for each bacterial species. pZB188 also contains a DNA fragment that confers resistance to tetracycline (i.e. a $Tc^r$-cassette). The first step in the construction of pZB188/aada-Gap*XylA*, was to remove the $Tc^r$-cassette from pZB188 and replace it with a DNA fragment that confers resistance to spectinomycin (i.e. $Spec^r$-cassette). To excise the $Tc^r$-cassette from pZB188, the plasmid was cut with ClaI and BssHII and the resulting large vector fragment was purified by agarose gel electrophoresis as described in more detail below. The $Spec^r$-cassette was generated by PCR using plasmid pHP15578 (Cahoon et al, (2003) Nature Biotechnology 21: 1082-1087) as a template and Primers 1 (SEQ ID NO:32) and 2 (SEQ ID NO:33). Plasmid pHP15578 contains the complete nucleotide sequence for the $Spec^r$-cassette and its promoter, which is based on the published sequence of the Tranposon Tn7 aadA gene (GenBank accession number X03043) that codes for 3' (9)-O-nucleotidyltransferase.

Primer 1 (SEQ ID NO: 32)

CTACTCATTTatcgatGGAGCACAGGATGACGCCT

Primer 2 (SEQ ID NO:33)

CATCTTACTacgcgtTGGCAGGTCAGCAAGTGCC

**[0100]** The underlined bases of Primer 1 (forward primer) hybridize just upstream from the promotor for the $Spec^r$-cassette (to nts 4-22 of GenBank accession number X03043), while the lower case letters correspond to a ClaI site that was added to the 5' end of the primer. The underlined bases of Primer 2 (reverse primer) hybridize about 130 bases downstream from the stop codon for the $Spec^r$-cassette (to nts 1002-1020 of GenBank accession number X03043), while the lower case letters correspond to an AflIII site that was added to the 5' end of the primer. The 1048 bp PCR-generated $Spec^r$-cassette was double-digested

with ClaI and AflIII, and the resulting DNA fragment was purified using the QIAquick PCR Purification Kit (Qiagen, Cat. No. 28104) and the vendor's recommended protocol. In the next step, plasmid pZB188 (isolated from *E. coli* SSC110 ($dcm^-$, $dam^-$) in order to obtain non-methylated plasmid DNA for cutting with ClaI, which is sensitive to dam methylation) was double-digested with ClaI and BssHII to remove the $Tc^r$-cassette, and the resulting large vector fragment was purified by agarose gel electrophoresis. This DNA fragment and the cleaned up PCR product were then ligated together, and the transformation reaction mixture was introduced into *E. coli* JM110 using chemically competent cells that were obtained from Stratagene (Cat. No. 200239). Note that BssHII and AflIII generate compatible "sticky ends", but both sites are destroyed when they are ligated together. Transformants were plated on LB medium that contained spectinomycin (100 μg/ml) and grown at 37 °C. A spectinomycin-resistant transformant that contained a plasmid with the correct size insert was identified by restriction digestion analysis with NotI, and the plasmid that was selected for further manipulation is referred to below as pZB188/aadA. A circle diagram of this construct is shown in Figure 6B.

**[0101]** In the next step, an *E. coli* xylose isomerase expression cassette was inserted between the NcoI and AclI sites of pZB188/aadA after cutting the latter with both enzymes, and purifying the large vector fragment by agarose gel electrophoresis. The ~2 Kbp DNA fragment that served as the *E. coli* xylose isomerase expression cassette was isolated from plasmid pZB4 by cutting the latter construct with NcoI and ClaI, and purifying the relevant DNA fragment by agarose gel electrophoresis. Plasmid pZB4 is described in detail in US 5514583, and a schematic representation of the *E. coli* xylose isomerase expression cassette P*gap*XylA (SEQ ID NO:34) is shown in the boxed diagram in Figure 6D.

**[0102]** The fragment containing the *E. coli* xylose isomerase expression cassette has an NcoI site and a ClaI site at its 5' and 3' ends respectively. As described in more detail in US 5514583, this fragment contains the strong, constitutive *Z.*

*mobilis* glyceraldehyde 3-phosphate dehydrogenase (GAP) promoter (nts 316-619), which is precisely fused to the complete open reading frame of the *E. coli* xylA open reading frame (nts 620-1942) that codes for xylose isomerase. It also contains the small stem-loop region that immediately follows the xylose isomerase stop codon (nts 1965-1999). The *E. coli* xylose isomerase expression cassette was inserted between the NcoI and AclI sites of pZB188/aadA in a standard ligation reaction. Note that ClaI and AclI generate compatible "sticky ends", but both sites are destroyed when they are ligated together. The ligation reaction mixture was then electroporated into *E. coli* SSC110 ($dcm^-$, $dam^-$) to obtain non-methylated plasmid DNA for subsequent transformation of *Z. mobilis*, and the transformed cells were plated on LB medium that contained 100 μg/ml of spectinomycin; growth was at 37 $^oC$. Spectinomycin-resistant tranformants that had a plasmid with a correct size insert were identified by restriction digestion analysis with NotI, NcoI and AclI. The plasmid that was selected for further manipulation and overexpression of *E. coli* xylose isomerase in the *Z. mobilis* ZW641 strain is referred to below as "pZB188/aadA-641Gap-XylA"; a circle diagram of this plasmid construct is shown in Figure 6C.

**[0103]** It is important to note that the nucleotide sequence of SEQ ID NO:34 is not identical to the nucleotide sequence that is described in SEQ ID NO:34 in co-owned and co-pending U.S. App. Pub.Nos. US20080286870and US20080187973, even though it corresponds to the same E. coli xylose isomerase expression cassette (P*gapXylA*). The DNA sequence disclosed in SEQ ID NO: 34 in the present work has a 1-bp deletion in the P*gap* that corresponds to nt 599 of SEQ ID NO:34 in U.S. App. Pub. Nos. US20080286870and US20080187973. The nucleotide sequence that was reported in the earlier patent applications was based on the published DNA sequence of the P*gap* for the Z. mobilis strain CP4 (Conway et al. J. Bacteriol. 169 (12):5653-5662 (1987)) and the promoter was not resequenced at that time. Recently, however, we have discovered that the P*gap* in pZB4 is also missing the same nucleotide, and the *E. coli* xylose isomerase expression cassette

(P*gapXylA*) that was used for all three patent applications was derived from this plasmid as noted above.

## EXAMPLE 6

## GENERATION OF AN *E. COLI* XYLOSE ISOMERASE EXPRESSION VECTOR THAT HAS THE SAME P*GAP* THAT DRIVES THE XYLA/B OPERON IN *Z. MOBILIS* ZW658 AND ZW801-4

**[0104]** Plasmid pZB188/aadA-801GapXylA is identical to pZB188-aada-641GapXylA (Figure 6C) but has a single nucleotide substitution in the P*gap* that corresponds to the G->T mutation that is present at position -189 in the P*gap* that drives expression of the *E. coli* XylA/B operon in ZW658. The same point mutation is also present in strains ZW800 and ZW801-4, which were sequentially derived from ZW658 as described below. The construction and characterization of ZW800 and ZW801-4 are describedin great detail in commonly owned and co-pending U.S. App. Pub.No. 11/862566. ZW800 is a derivative of ZW658 which has a double-crossover insertion of a spectinomycin resistance cassette in the sequence encoding the glucose-fructose oxidoreductase (GFOR) enzyme that inactivates this activity. ZW801-4 is a derivative of ZW800 in which the spectinomycin resistance cassette was deleted by site-specific recombination leaving an in-frame stop codon that prematurely truncates the protein. None of these manipulations altered the nucleotide sequence of the mutant P*gap* promoter that drives the XylA/B operon in ZW658. Thus, the “801GAP promoter” refers to the promoter sequence that is present in the following strains: ZW658, ZW800, and ZW801-4.

**[0105]** The steps and plasmid intermediates that were used to generate pZB188/aadA-801GapXylA are described below in chronological order starting with the plasmid pMOD-Linker.

*Construction of pMOD-Linker*

**[0106]** The precursor for plasmid pMOD-Linker was the pMOD™-2<MCS> Transposon Construction Vector (Cat. No. MOD0602) that is commercially available from EPICENTRE®. As shown in Figure 7A, pMOD™-2<MCS> has an

ampicillin resistance gene (ampR), an *E. coli* origin of replication (ori), and a multi-cloning site that is situated between the two mosaic ends (ME) that Tn5 transposase interacts with. The first step in the construction of pMOD-Linker was to remove the original multi-cloning site in pMOD2-<MCS> and replace it with a new multi-cloning site that has unique restriction sites for AsiSi, FseI and SbfI. This was done by cutting the plasmid with EcoRI and HindIII and purifying the large (about 2.5 Kbp) vector fragment by agarose gel electrophoresis. The new multi-cloning site was then generated by annealing together two synthetic oligonucleotides, Linker B (SEQ ID NO:35) and Linker T (SEQ ID NO:36) that were both phosphorylated at their 5' end.

Linker B (SEQ ID NO:35):
aattCTACCTGCAGGAGTAGGCCGGCCATGAGCGATCGCA
Linker T (SEQ ID NO:36):
agctTGCGATCGCTCATGGCCGGCCTACTCCTGCAGGTAG

**[0107]** These oligonucleotides are complimentary to each other, and when annealed together form a double stranded linker that has single-stranded overhangs at both ends (lower case letters), which allow the DNA fragment to be ligated between the EcoRI and HindIII sites of the large pMOD™-2<MCS> vector fragment described above. As noted above this synthetic linker also contains three unique restriction sites (AsiSi, FseI and SbfI) that can be used for subsequent cloning steps. The SbfI site is underlined with a thin line, the FseI site is underlined with a thick line and the AsiSI site is underlined with two thin lines. Linker B and Linker T were annealed together and the resulting DNA fragment was inserted between the EcoRI and HindIII sites of pMOD™-2<MCS> in a standard ligation reaction. The ligation reaction mixture was used to transform *E. coli* DH10B and the transformed cells were plated on LB media that contained 100 μg/ml of ampicillin. Plasmid DNA was then isolated from a representative ampicillin-resistant colony that contained the new multi-cloning

site. A circle diagram of the resulting plasmid construct (referred to below as "pMOD-Linker") is shown in Figure 7B.

Construction of pMOD-Linker-Spec

**[0108]** A DNA fragment that confers resistance to spectinomycin ($Spec^r$) and has a wild type loxP site at both ends was inserted between the AsiSI and FseI sites of the pMOD-Linker construct described above. The source of the loxP-flanked $Spec^r$ cassette was plasmid pLDH-Sp-9WW (Figure 8), which is described in great detail in U.S. App. No. 11/862566. In the first step, MOD-Linker plasmid DNA was sequentially digested with FseI and AsiSI, and the large vector fragment was purified using a DNA Clean & ConcentratorTM-5 spin column kit that was purchased from Zymo Research Corporation (Cat. No. DO4003). Next, plasmid pLDH-Sp-9WW was also double-digested with the same two enzymes and the small (about 1.1 Kbp) DNA fragment that contained the loxP-flanked $Spec^r$ cassette was purified by agarose gel electrophoresis. The two DNA fragments of interest were then ligated together, and the transformation reaction mixture was introduced into *E. coli* DH10B using electroporation. Transformants were plated on LB media that contained ampicillin (100 μg /ml) and spectinomycin (100 μg /ml) and growth was at 37 °C. Plasmid DNA was then isolated from one of the ampicillin-resistant colonies that contained a DNA fragment with the correct size and this was used for subsequent manipulations. A circle diagram of this construct (referred to below as "pMOD-Linker-Spec") is shown in Figure 7C.

Construction of pMOD-Linker-Spec-801GapXylA and pMOD-Linker-Spec-641GapXylA

**[0109]** A DNA fragment that contains the entire P*gap,* the XylA coding region, and the stem-loop region that is between the XylA and XylB open reading frames was PCR-amplified from ZW801-4 using Primers 3 and 4 (SEQ ID NOs:37 and 38, respectively) and resuspended cells as a template. As already noted, DNA sequence analysis has shown that ZW801-4 has the same G->T point mutation

at position -189 in the P*gap* promoter that drives the expression of the integrated *E. coli* XylA/B operon as ZW658 and that the P*gap* in both strains are identical.

Primer 3 (SEQ ID NO:37)
TCACTCATggccggccGTTCGATCAACAACCCGAATCC
Primer 4 (SEQ ID NO:38)
CTACTCATcctgcaggCCGATATACTTATCGATCGTTCC

**[0110]** The underlined bases of Primer 3 (forward primer) hybridize to the first 22 bases of the P*gap* (and to nts 316-337 of SEQ ID NO:34, while the lower case letters correspond to an FseI site that was added to the 5' end of the primer. The underlined bases of Primer 4 (reverse primer) hybridize just downstream from the stem-loop region that is after the XylA stop codon (and to the last 12 nts of SEQ ID NO:34), while the lower case letters correspond to an SbfI site that was added to the 5' end of the primer.

**[0111]** The PCR product was double-digested with FseI and SbfI, and purified using a DNA Clean & ConcentratorTM-5 spin column kit that was purchased from Zymo Research Corporation (Cat. No. DO4003). Next, plasmid pMOD-Linker-Spec was cut with the same two enzymes and the resulting large vector fragment was purified using the same procedure. The two DNA fragments of interest were then ligated together, and the transformation reaction mixture was introduced into *E. coli* DH10B using electroporation. The cells were plated on LB media that contained ampicillin (100 μg /ml) and spectinomycin (100 μg /ml) and growth was at 37 $^{o}$C. Transformants that contained a plasmid with a correct size insert were identified by PCR using Primers 3 and 4 and resuspended colonies as a template ("colony PCR"). The plasmid that was selected for further manipulation is referred to below as pMOD-Linker-Spec-801GapXylA, and a circle diagram of this construct is shown in Figure 9.

**[0112]** The same steps described above were used to generate another plasmid that is referred to below as "pMOD-Linker-Spec-641GapXylA", except the template that was used for PCR-amplification of the P*gap*-XylA gene DNA fragment was a cell suspension of ZW641. pMOD-Linker-Spec-641GapXylA and pMOD-Linker-Spec-801 GapXylA are identical except for the G->T substitution in the P*gap* that distinguishes ZW658 (and ZW801-4) from ZW641.

Construction of pZB188-aadA-801GapXylA

**[0113]** As described in the first paragraph of Example 6, pZB188-aadA-801GapXylA is an *E. coli* Xylose Isomerase expression vector *for Z. mobilis* that is identical to pZB188-aadA-641GapXylA, but it has the same G->T substitution in the P*gap* that drives expression of the integrated Pgap-XylA/B operon in ZW658 (and ZW801-4). To construct this plasmid, pMOD-Linker-Spec-801GapXylA (Figure 10A) was double digested with MluI and SalI and the smaller DNA fragment (about1100 bp) was purified using agarose gel electrophoresis and the Zymoclean Gel DNA Recovery Kit (catalog #D4001, Zymo Research). This fragment contains the P*gap* G->T substitution and part of the XylA ORF and was used to replace the corresponding fragment in pZB188-aadA-641GapXylA (Figure 10B), after cutting the latter construct with the same two enzymes and purifying the large vector fragment by agarose gel electrophoresis. The two fragments of interest were then ligated together and the ligation reaction mixture was introduced into *E. coli* DH10B using electroporation. Transformants were plated on LB media that contained spectinomycin (100 μg/ml) and growth was at 37 °C. Plasmid DNA was isolated from a spectinomycin-resistant colony and the presence of the P*gap* promoter G->T substitution was confirmed by DNA sequence analysis. The plasmid used for subsequent manipulations, ("pZB188-aadA-801GapXylA") is shown in Figure 10C.

## EXAMPLE 7

## OVEREXPRESSION OF *E. COLI* XYLOSE ISOMERASE IN ZW641

**[0114]** The enzyme activity measurements in Table 4 show that xylose isomerase and xylulokinase activities increased dramatically during the transition from ZW641 to ZW658. To test the hypothesis that xylose isomerase is the rate-limiting enzyme for growth on xylose in ZW641, the enzyme was overexpressed in this strain using the multicopy plasmid, pZB188/aadA-641GapXylA (Fig. 6C). The control for this experiment was ZW641 transformed with the multicopy plasjmid pZB188/aadA, which lacks the P*gap-E. coli* xylose isomerase expression cassette (Fig. 6B). The construction of both of these plasmids is described in Example 5, and the transformation protocol was essentially as described in Example 5 of commonly owned and co-pending U.S. App. Pub. No. US20080187973. Briefly, non-methylated plasmid DNA (isolated from from *E. coli* SSC110, which is a $dcm^-$ and $dam^-$ strain) was introduced into ZW641 using electroporation, and the transformed cells were plated on LB media that contained 200 μg/ml spectinomycin. After a 48-hr growth period at 30 $^oC$ under anaerobic conditions, three primary transformants were randomly selected for each plasmid, and these were patched (transferred) onto agar plates that contained the same growth media for further characterization.

**[0115]** Figure 11 shows growth curves (OD600 versus time) in xylose-containing media for the three strains that harbored the 641P*gap-E. coli* xylose isomerase expression plasmid (X1, X2 and X2) and the three strains that harbored the control plasmid (C1, C2 and C3). This experiment was performed at 30 $^oC$ in shake flasks (5-ml cultures in 15-ml tubes at 150 rpm), and the growth media was mRM3-X10 (10 g/L yeast extract, 2 g/L $KH2PO_4$, 1 g/L $MgSO_4$ and 100 g/L xylose) that also contained spectinomycin (200 μg/ml). The cultures were started with a loop of cells from the patched plate described in the above paragraph and the initial OD600 in each case was about 0.13. Similar to ZW641, the three strains with the control plasmid barely grew on xylose. In marked contast, both the rate and extent of growth (final OD600 values) on xylose were dramatically improved when ZW641 was transformed with the 641P*gap-E. coli* xylose isomerase expression plasmid, pZB188/aadA-641GapXylA. Since all three

strains that had this plasmid behaved the same in the experiment that is shown in Fig. 11, only the X1 strain and C1 strain were subjected to further characterization.

**[0116]** Figure 12 shows a side-by-side comparison of ZW641, ZW658, X1 and C1 in the same xylose containing growth media without spectinomycin. The conditions for this experiment were identical to those described above but the 20-ml cultures were grown in 50-ml tubes and the initial OD600s were about 4-fold lower ( 0.035). The growth curves shown in Figure 12A are plotted on a linear scale (OD600 versus Time), while Figure 12B shows the same experimental data plotted on a logarithmic scale (logOD600 versus Time) in order to compare exponential growth rates. It is apparent from this experiment that the exponential growth rate of X1 is almost as fast as the xylose-adapted strain ZW658, and that this strain grows much better on xylose than the parent strain ZW641 with or without the control plasmid. Thus, high expression of xylose isomerase in ZW641 (driven by a 641P*gap* promoter from a multicopy plasmid) has a similar effect on growth on xylose as the increase in xylose isomerase activity had on ZW658 (shown in Table 4). Although the final biomass yield for X1 is about 2-fold lower than that obtained with ZW658, it is clear from this data that the rate-limiting enzyme for growth on xylose in ZW641 is xylose isomerase. The experiments shown in Figures 11 and 12 further suggest two other interesting possibilities: (1) that the large increase in xylose isomerase activity that occurred during the transition from ZW641 to ZW658 (Table 4) was largely responsible for the better growth on xylose that occurred during the "xylose adaption" process; and 2) that the increase in xylose isomerase activity may have resulted from the G->T substitution in the P*gap* promoter that drives expression of the chromosomally-integrated P*gap*-XylA/B operon that is present in ZW658.

## EXAMPLE 8

### TRANSPOSON-MEDIATED INTEGRATION OF E. COLI XYLOSE ISOMERASE IN ZW641

**[0117]** ZW641 and two plasmid constructs (pMOD-Linker-Spec-801GapXylA and pMOD-Linker-Spec-641GapXylA) were used to test the hypothesis that the P*gap*

promoter with the G->T substitution that drives expression of the integrated XylA/B operon in ZW658 (henceforth referred to as the "801GAP promoter") is stronger than the corresponding promoter in ZW641 (henceforth referred to as the "641GAP promoter"). ZW641 was selected for these experiments since it's barely able to grow on xylose, and because overexpression of xylose isomerase in this strain results in faster growth on xylose (Example 7, Figs. 11 and 12). The basic idea was to introduce an extra copy of the *E. coli* xylose isomerase gene (driven by the 641GAP promoter or the 801GAP promoter) into the chromosome of ZW641 and see which construct would result in the fastest growth on xylose. Chromosomal integration of the two chimeric genes was accomplished using Epicentre's transposome technology.

**[0118]** As already indicated, pMOD-Linker-Spec-641GapXylA and pMOD-Linker-Spec-801GapXylA are identical plasmids except for the G->T point mutation that is present in the P*gap* promoter in the latter construct. The transposable element used for random insertion into DNA in both cases consisted of the two 19-bp mosaic ends (MEs) and the entire DNA fragment that is sandwiched between them. As shown in Fig. 9, this element, which is referred to as the transposon, contains a spectinomycin-resistance cassette (Spec$^r$) and a downstream P*gap*-*E. coli* xylose isomerase expression cassette. The protocol that was used to form the transposomes was essentially the same as that described in Epicentre's instruction manual for the EZ::TN$^{TM}$pMOD$^{TM}$-2<MCS> Transposn Construction Vector (Cat. No. MOD0602). The 8-μL reaction contained 1.5 μL of 5'-phosphorylated, blunt-ended transposon DNA that was free of $Mg^{++}$ ions (about 250 ng/ μL), 4 μL of Epicentre's EZ::TN Transposase and 2.5 μL of 80% (v/v) glycerol. The control transposome reaction mixture was identical but 4 μL of sterile water was substituted for the transposase. The reactions were incubated at room temperature for 30 min and were then transferred to 4 $^oC$ for a 2- to 7-day incubation period that is required for the slow isomerization step, which results in the formation of the active transposmome; using this procedure the transposomes are stable for at least 3 months at -20 $^oC$.

**[0119]** The transposomes were electroporated into ZW641 essentially using the same transformation protocol that is described in US 5,514,583. Briefly, the 40-μL transformation reactions contained about $10^{10}$ cells/ml in 10% (v/v) glycerol, 1 μL of Epicentre's TypeOne™ Restriction Inhibitor (Cat. No. TYO261H) and 1 μL of the control or transposome reaction mixture. The settings for the electroporator were 1.6 kv/cm, 200 Ω, and 25 μF, and the gap width of the cuvette was 0.1 cm. Following electroporation, the transformation reactions were diluted with 1.0 ml of MMG media (50 g/L glucose, 10 g/L yeast extract, 5 g/L of tryptone, 2.5 g/L of $(NH_4)_2SO_4$, 0.2 g/L $K_2HPO$, and 1 mM $MgS0_4$) and the cells were allowed to recover for about3 hours at 30°C. The cells were then harvested by centrifugation at room temperature (13, 000 X g, 5 min) in sterile 1.5-ml microfuge tubes and the supernatant was carefully removed. Cell pellets were resuspended in 200 μL of liquid MMG media and a 100-μL aliquot of each cell suspension was plated on MMG media that contained 1.5% agar and 200 μg/ml of spectinomycin. After a 72-hr incubation period at 30 °C under anaerobic conditions, 3 colonies were on the control plate, 13 colonies were on the 641GapXylA transposome plate and 18 colonies were on the 801GapXylA transposome plate. Six colonies from both transposome plates were randomly selected for further characterization, and these were patched onto agar plates that contained MMX media and 200 μg/ml of spectinomycin; the growth conditions were as described above. MMX media is the same as MMG media, but contains 50 g/L of xylose instead of glucose. After a second round of growth on a fresh MMX plus spectinomycin plates, the six strains that grew the best on xylose (three for each transposome) were used for the experiment described below.

**[0120]** Figure 13A shows linear growth curves for the three ZW641 strains that were obtained with the 641Gap-XylA transposome (#6-1, #6-3 and #6-5) and the three that received the 801Gap XylA transposome (#8-2, #8-4 and #8-5) in

xylose-containing media. The same data is plotted on a log scale in Fig. 13B. This experiment was performed at 30 $^{o}$C in shake flasks (7-ml cultures in 15-ml tubes at 150 rpm), and mRM3-X10 (10 g/L yeast extract, 2 g/L $KH2PO_4$, 1 g/L $MgSO_4$ and 100 g/L xylose) was the growth media. The cultures were started with a loop of cells from the patched plate described above and the initial ODs were very similar (about0.02-0.03). The control for this experiment was the xylose-adapted strain ZW658, which has the G->T substitution in the P*gap* that drives the chromosomally-integrated *E. coli* XylA/B operon.

**[0121]** Similar to the parent strain (ZW641) the three strains that had an extra chromosomally-integrated copy of the 641GapXylA expression cassette grew very poorly in xylose-containing media, although it was apparent that there were some minor improvements in both the growth rate and biomass yield (OD600), especially for strain #6-5 (compare Fig. 12A and Fig. 13A). In contrast, all three of the strains that were obtained with the 801GapXylA transposon grew much better on xylose than the parent strain (Fig 13A and 13B). In fact, two of the transformants (#8-4 and #8-5) grew almost as well on this sugar as ZW658 and the ZW641 transformants that harbored the multi-copy plasmid pZB188/aadA-GapXylA, which contains a 641GapXylA expression cassette (compare Fig. 12 and Fig. 13). Since transposition is a random event and all six strains have the 641GapXylA or 801GapXylA expression cassette inserted at different locations in the chromosome, differences in foreign gene expression that were observed in this experiment using the same transposome are likely to be due to positional effects. For example, position effects may account for the better growth of #6-5 than of #6-1 and #6-3, and for the poorer growth of #8-2 than of #8-4 and 8-5. Nevertheless, despite the small size of the population that was analyzed, the results that are shown in Fig. 13 strongly support the notion that the G->T mutation that is present in the P*gap* promoter that drives the *E. coli* XylA/B operon in ZW658 and ZW801-4 is responsible for the higher xylose isomerase activity and better growth on xylose that is observed with these strains, compared to the parent strain ZW641.

## EXAMPLE 9

## ENZYME ACTIVITY AND SEQUENCE COMPARISON OF THE TRANSGENE GAP PROMOTER REGIONS OF INDEPENDENTLY ADAPTED STRAINS OF XYLOSE UTILIZING *Z. MOBILIS*

**[0122]** Since strain 8b (Example 1 and US App. Pub. No. 20030162271) was obtained using a similar course of gene introduction and strain adaptation as was ZW658, the transgene activities of the pentose phosphate pathway and the sequence of the P*gapxylAB* operon were also compared in partially and more fully adapted strains of this independent strain production. Enzyme activities for products of the P*gapxylAB* operon in a partially adapted strain 8XL4 and the final adapted strain 8b were measured using the techniques described in General Methods and the results expressed as μmoles product/mg protein/minute are shown in Table 5.

Table 5. Enzyme activities in different xylose-utilizing adapted *Z. mobilis* strains

| Strain | Xylose isomerase | Xyulose kinase |
|---|---|---|
| 8XL4 | 0.027+/- 0.004 | 1.10 +/- 0.41 |
| 8b | 0.142 +/- 0.057 | 5.76 +/- 0.43 |

**[0123]** As with the adaptation that occurred when the strains preceding ZW658 picked up mutations that allowed enhanced growth on xylose, strain 8b had increased activity for products of both genes in the xylAB operon over its predecessor strain 8XL4. Once again the increase in measured enzyme activity was about five fold increased over the less adapted strain.

**[0124]** The P*gap* directing expression of the xylAB operon was sequenced in the 8b and 8XL4 strains. A PCR fragment was prepared using a forward PCR primer (GAP-F8; SEQ ID NO:39) from the 5' end of the promoter and a reverse primer from the xylA coding region (XylAB851R; SEQ ID NO:5). The resulting PCR product was sequenced using primers GAP-F8, XylAB449R, and XylAB851R (SEQ ID NOs:39, 41, and 40). The promoter sequence from ZW8XL4 is given in

SEQ ID NO:3 and that from 8b in SEQ ID NO:5. These promoter sequences also both had the one difference with the published sequence of the P*gap* of strain CP4 as in the P*gap* of the xylAB operon in ZW641 and ZW658. In addition to these common changes there was also a single base pair difference between the ZW641 and ZW658 *Pgap* sequences. While the G to T change at -189 to the start ATG was not present in the comparison of 8XL4 and 8b, a C to T change did occur at position -89 with respect to the start ATG.

**[0125]** As with the promoter sequence of the P*gapxylAB* operon in strain ZW658, the promoter sequence of the P*gapxylAB* operon in strain 8b changed during adaptation to a new sequence which allowed production of more of the protein from the coding regions under its control than did the sequence of the same promoter from the partially adapted strain.

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**[0126]** Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification , they are to be interpreted as specifying the presence of the stated features, integers, steps or components referred to, but not to preclude the presence or addition of one or more other feature, integer, step, component or group thereof.

**[0127]** Further, any prior art reference or statement provided in the specification is not to be taken as an admission that such art constitutes, or is to be understood as constituting, part of the common general knowledge in Australia.

## Table 3

HMMER2.0 [2.3.2][1]
NAME brenda_xylA3_seqs-con[2]
LENG 455[3]
ALPH Amino[4]
MAP yes[5]
COM hmmbuild brenda-xyla3.hmm brenda_xylA3_seqs-con.aln[6]
COM hmmcalibrate brenda-xyla3.hmm[7]
NSEQ 32[8]
DATE Wed Mar 12 21:55:22 2008[9]
XT -8455 -4 -1000 -1000 -8455 -4 -8455 -4
NULT -4 -8455[10]
NULE 595 -1558 85 338 -294 453 -1158 197 249 902 -1085 -142 -21 -313 45 531 201 384 -1998 -644[11]
EVD -379.726868 0.105452[12]

| HMM | A | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|
| | m->m | m->i | m->d | i->m | i->i | d->m | d->d | b->m |
| | -152 | * | -3322 | | | | | |
| 1(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -383 | -8312 | -2119 | -894 | -1115 | -701 | -1378 | -152 |
| 2(I) | -652 | -1642 | -4507 | -4008 | 395 | -3998 | -3165 | 3120 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| 3(D) | -60 | -2403 | 1750 | 355 | -2717 | 573 | -526 | -2474 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| 4(K) | -1273 | -2543 | -1243 | -600 | -2936 | -2186 | -668 | -2589 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| 5(I) | -2454 | -1957 | -5121 | -4804 | -2568 | -4911 | -4887 | 3182 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| 6(Q) | -232 | -2237 | -616 | 690 | -2557 | -420 | -400 | -2306 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| 7(Y) | -4607 | -3566 | -5021 | -5359 | 2754 | -4894 | -1132 | -3491 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| 8(E) | -1557 | -2509 | -804 | 3252 | -3197 | -2167 | -1540 | -2531 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| 9(G) | -4079 | -3917 | -4766 | -5131 | -5608 | 3825 | -4746 | -6295 |
| - | -149 | -500 | 233 | 44 | -381 | 398 | 108 | -625 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -284 | -2499 | -8980 | -602 | -1552 | -1952 | -431 | * |
| | | | | | | | | |
| 10(K) | -211 | -2265 | -699 | -143 | -2591 | -1802 | -444 | -2325 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 11(K) | -238 | -2256 | 1519 | 758 | -2576 | -602 | -412 | -2328 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 12(S) | -24 | -1368 | -1330 | -776 | -1457 | -2006 | -777 | -219 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 13(K) | -815 | -2291 | 332 | 381 | -2610 | -1764 | -439 | -2362 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 14(N) | -112 | -2600 | -523 | 381 | -2952 | -1931 | -753 | -2702 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 15(P) | -67 | -1766 | -1443 | -999 | -2363 | 1282 | -1134 | -1992 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 16(L) | -1558 | -1364 | -253 | -3026 | 1709 | -3054 | -1523 | -618 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1508 | -625 | * |
| | | | | | | | | |
| 17(A) | 2539 | -1340 | -2407 | -1923 | -114 | 254 | -1611 | -1400 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 18(F) | -3970 | -3283 | -4844 | -4992 | 4163 | -4617 | -1220 | -3051 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 19(K) | -1512 | -2771 | -1507 | -818 | -3232 | -2385 | 2267 | -2858 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 20(Y) | -3420 | -2864 | -4689 | -4633 | 1930 | -4361 | 3299 | -2576 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 21(Y) | -3783 | -3746 | -2646 | -265 | -826 | -3845 | -1884 | -3929 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 22(N) | -2403 | -4115 | 2160 | -610 | -4589 | -2333 | -1803 | -4558 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 23(P) | 1749 | -1901 | -2474 | -2677 | -4084 | -2051 | -2900 | -3873 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 24(E) | -1269 | -2810 | 2039 | 2488 | -3105 | -1980 | -824 | -2879 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 25(E) | -1569 | -3030 | -675 | 2974 | -3390 | -2199 | -979 | -3118 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 26(V) | -940 | -874 | -2689 | 438 | 215 | -2419 | -1259 | 1264 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1811 | -484 | * |
| | | | | | | | | |
| 27(I) | -2373 | -1909 | -4992 | -4608 | -2445 | -4701 | -4324 | 2910 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8005 | -9047 | -894 | -1115 | -1147 | -867 | * |
| | | | | | | | | |
| 28(M) | 297 | -2163 | 527 | 293 | -2408 | 1145 | -557 | -2107 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8107 | -9149 | -894 | -1115 | -623 | -1512 | * |
| | | | | | | | | |
| 29(G) | -858 | -2502 | 613 | -529 | -2936 | 2600 | -948 | -2664 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8252 | -9294 | -894 | -1115 | -533 | -1695 | * |
| | | | | | | | | |
| 30(K) | -1648 | -2125 | -2186 | -1533 | -1645 | -2748 | 734 | -1787 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 31(T) | -1046 | -2501 | -897 | 404 | -2830 | -2011 | -653 | -2571 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 32(M) | -551 | -1875 | -3895 | -3452 | -1627 | -3127 | -2589 | -1019 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 33(K) | 127 | -2511 | -888 | 1379 | -2865 | -2030 | -726 | -2605 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 34(D) | -2246 | -3964 | 2730 | 2201 | -4210 | -2482 | -1633 | -4052 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 35(W) | -307 | -2255 | -1047 | 1375 | -2417 | -2110 | 3282 | -2137 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 36(L) | -2283 | -2311 | 1522 | -2528 | 661 | -3412 | 1226 | -1601 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 37(R) | -3325 | -3893 | -4311 | -2623 | -4835 | -3766 | 1845 | -4080 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 38(F) | -5308 | -4420 | -5610 | -5948 | 4547 | -4921 | -3048 | -4656 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 39(S) | 2011 | -1975 | -4184 | -4245 | -4465 | -522 | -3670 | -4249 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 40(V) | -389 | -1555 | -4211 | -3620 | 2221 | -3520 | -2467 | 827 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 41(A) | 2968 | 1546 | -4577 | -4780 | -4439 | 1767 | -3862 | -4189 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 42(Y) | -4757 | -3801 | -5352 | -5629 | 2454 | -5193 | -1481 | -3601 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 43(W) | -6178 | -4896 | -5848 | -6204 | -4109 | -4952 | -4743 | -6650 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 44(H) | -2390 | -2940 | -3006 | -3167 | -3361 | -3029 | 4890 | -4377 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -383 | -8312 | -2119 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 45(T) | -1111 | -1722 | -3459 | -3693 | -4071 | -1979 | -3313 | -3836 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -254 | -2629 | * |
| | | | | | | | | |
| 46(F) | -2728 | -2359 | -5136 | -4558 | 3354 | -4572 | -3468 | 910 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 47(C) | -1098 | 2971 | 1338 | -866 | -2368 | 1340 | -1058 | -2007 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 48(W) | 1080 | -2296 | -913 | 535 | -2548 | 415 | -675 | -2251 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 49(D) | -994 | -2466 | 1756 | 978 | -2785 | -1962 | -625 | -2536 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 50(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 51(G) | -321 | -2099 | -3782 | -3944 | -3733 | 3298 | -3507 | -3250 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 52(R) | 877 | -2295 | -923 | -374 | -2567 | -29 | -672 | -2273 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 53(D) | -4886 | -4905 | 4186 | -3339 | -5949 | -4221 | -4195 | -6666 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 54(P) | -358 | -1704 | -2202 | -1724 | -1995 | -2408 | -1572 | -1504 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 55(F) | -5308 | -4420 | -5610 | -5948 | 4547 | -4921 | -3048 | -4656 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 56(G) | -4740 | -4445 | -5364 | -5738 | -6131 | 3840 | -5265 | -6903 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 57(D) | 674 | -2424 | 2070 | 271 | -2717 | 297 | -669 | -2448 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 58(G) | 2216 | -2048 | -3999 | -4272 | -4632 | 2814 | -3817 | -4443 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 59(T) | -552 | -1875 | -3402 | -3173 | -3341 | -2281 | -2846 | -2926 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 60(M) | 1157 | -1590 | -1704 | -1125 | 650 | -2335 | -1036 | -1217 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 61(Q) | 506 | -2444 | 274 | 1073 | -701 | -1222 | -615 | -365 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 62(R) | 716 | -1736 | -2244 | -1683 | 752 | -2703 | -1193 | -1344 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 63(P) | -625 | -2530 | 60 | -1072 | -2925 | -2398 | -1624 | -2563 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -90 | -8312 | -4120 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 64(W) | -4762 | -4593 | 2301 | -3636 | -3451 | -4258 | -3708 | -5821 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -130 | -8229 | -3598 | -894 | -1115 | -1121 | -889 | * |
| | | | | | | | | |
| 65(D) | -1309 | -2840 | 2011 | 494 | -3135 | -2037 | -873 | -2906 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -2311 | -8107 | -331 | -894 | -1115 | -628 | -1503 | * |
| | | | | | | | | |
| 66(Y) | -635 | -882 | -1209 | -779 | 422 | -1778 | -110 | -454 |
| - | -149 | -500 | 234 | 43 | -381 | 399 | 106 | -626 |
| - | -1029 | -988 | -7468 | -137 | -3461 | -571 | -1614 | * |
| | | | | | | | | |
| 67(H) | -9 | -2243 | 374 | 1170 | -2564 | 95 | 1478 | -2314 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 68(Y) | -804 | -1603 | -77 | -470 | -287 | 1328 | -615 | -1330 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 69(T) | -167 | -2183 | 188 | -187 | -2570 | 677 | -524 | -2303 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 70(D) | -1090 | -2605 | 2512 | 741 | -2908 | 391 | 837 | -2674 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -262 | -7937 | -2624 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 71(P) | 1251 | -2101 | -546 | 596 | -2491 | -1704 | -497 | -2212 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -10 | -7686 | -8728 | -894 | -1115 | -1200 | -825 | * |
| | | | | | | | | |
| 72(M) | -1983 | -1721 | -4201 | -671 | -1027 | -3652 | -2508 | 473 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 73(D) | 325 | -3516 | 2804 | 2034 | -3761 | -2131 | -1242 | -3593 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 74(M) | -162 | 687 | -1246 | 48 | -1449 | -1992 | -713 | 457 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 75(A) | 3609 | -2502 | -4177 | -4484 | -4584 | -2730 | -3982 | -4412 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 76(K) | -1027 | -2025 | -1083 | 321 | -188 | -2040 | 1105 | -1850 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 77(A) | 2085 | -2164 | -961 | -400 | -2453 | -1963 | -598 | -2122 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 78(R) | -3027 | -3580 | -3980 | -2342 | -4547 | -3455 | -1266 | -3796 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 79(V) | 1224 | -1553 | -4145 | -3706 | -1862 | -3327 | -2889 | 214 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -254 | -2629 | * |
| | | | | | | | | |
| 80(D) | -493 | -3116 | 2767 | 1555 | -3391 | -2262 | -1166 | -3139 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 81(A) | 2284 | -1391 | -2040 | 1144 | -1397 | -2450 | -1253 | -149 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 82(A) | 2251 | -1006 | -3057 | -2446 | 931 | -297 | 873 | 186 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 83(F) | -2224 | -2250 | -4424 | -4264 | 3927 | -1145 | -2398 | -1376 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 84(E) | -2532 | -3829 | -1248 | 3330 | -4180 | -2869 | 2424 | -3974 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 85(F) | -1403 | -1396 | -2948 | -2272 | 3286 | -2799 | -1493 | 1022 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 86(F) | -317 | 889 | -3643 | -3012 | 2565 | -2871 | -1711 | -515 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 87(E) | 1138 | -2517 | -22 | 2128 | -2834 | -1986 | 953 | -2586 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 88(K) | -3061 | -4130 | -1699 | 1837 | -4807 | -3224 | -1891 | -4412 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 89(L) | -3259 | -2823 | -5615 | -5021 | -1437 | -5150 | -3961 | 745 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 90(G) | -1754 | -3293 | 758 | -600 | -3610 | 2854 | 295 | -3395 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 91(V) | 1814 | -1770 | -4355 | -3892 | -2144 | -3716 | -3087 | 1744 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 92(P) | -1176 | -2665 | 795 | 1462 | -2975 | -2054 | 1351 | -2732 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 93(Y) | -3660 | -3259 | -4548 | -4414 | 1974 | 1637 | -1482 | -3075 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 94(F) | -1766 | -1543 | -3984 | -3431 | 2846 | -3263 | -1648 | 658 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 95(C) | 367 | 5085 | -4219 | -4291 | -4096 | -2272 | -3593 | -3763 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 96(F) | -4420 | -3702 | -5536 | -5637 | 4337 | -5230 | -2140 | -2246 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 97(H) | -5506 | -4764 | -5029 | -5331 | -4362 | -4732 | [redacted] | -6634 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 98(D) | -4886 | -4905 | 4186 | -3339 | -5949 | -4221 | -4195 | -6666 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 99(R) | -1078 | -2255 | 1071 | 345 | -2468 | -2070 | -762 | 246 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 100(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 101(D) | -4886 | -4905 | [redacted] | -3339 | -5949 | -4221 | -4195 | -6666 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 102(I) | -3148 | -2630 | -5718 | -5282 | -1996 | -5478 | -4785 | 2752 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 103(A) | 2332 | 880 | -3499 | -2885 | 155 | -2753 | -1687 | 1413 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 104(P) | -672 | -3716 | 211 | -35 | -4479 | -2521 | -1993 | -4330 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 105(E) | -1113 | -2563 | 755 | 2544 | 1516 | -2031 | -733 | -2609 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 106(G) | 299 | -2657 | 34 | -2104 | -4774 | 3464 | -2959 | -4642 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 107(D) | 940 | -2485 | 1893 | 127 | -2803 | -1971 | 844 | -2554 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 108(T) | -1565 | -2482 | 739 | -1528 | -3930 | -2299 | -2089 | -3698 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 109(L) | 183 | -1297 | 530 | -2212 | -1245 | -2705 | -1605 | 1003 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 110(K) | 640 | -2464 | -846 | 531 | -2786 | -634 | -624 | -2534 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 111(E) | -2632 | -4118 | -726 | 3650 | -4688 | -2682 | -2003 | -4498 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 112(T) | -1378 | -1595 | -2330 | -1769 | 970 | -2639 | -1269 | -1211 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -383 | -8312 | -2119 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 113(N) | -791 | -1832 | 50 | 1047 | -305 | -1841 | -525 | -703 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 114(K) | 1001 | -2246 | -618 | 864 | -2565 | -1750 | 758 | -2313 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 115(N) | -2127 | -2452 | -2097 | -2034 | -660 | -2951 | -1368 | -198 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -1952 | -431 | * |
| | | | | | | | | |
| 116(L) | -3492 | -2924 | -5834 | -5257 | 789 | -5550 | -4102 | 1027 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -254 | -2629 | * |
| | | | | | | | | |
| 117(D) | 352 | -2696 | 2970 | 584 | -3009 | -2082 | -807 | -2762 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 118(Q) | -379 | -2417 | -845 | 1254 | -2722 | -784 | 874 | -200 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 119(M) | -1906 | -1631 | -4282 | -3715 | -1632 | -3659 | 1153 | 2809 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 120(V) | -1675 | -1428 | -4046 | -3465 | -1535 | -3381 | -2355 | 1345 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 121(D) | 893 | -2550 | 2186 | 1147 | -2867 | 374 | -691 | -2620 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 122(M) | -1064 | -1348 | -380 | -1330 | -160 | -875 | 767 | 37 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 123(I) | 624 | -2059 | -4727 | -4136 | 2069 | -4088 | -2966 | 2565 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 124(K) | 164 | -2420 | -870 | 864 | -2723 | -1981 | -637 | -2456 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 125(Q) | -1086 | -2570 | 755 | 2102 | -2887 | -239 | -702 | -2641 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 126(K) | 1143 | -2138 | -1047 | -491 | -2334 | -1268 | -731 | -1997 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 127(M) | -3159 | -2791 | -5237 | -4626 | -1375 | -4924 | -3658 | 241 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 128(K) | 531 | -2467 | 1672 | 432 | -2787 | -1962 | 842 | -2538 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 129(E) | 608 | -2501 | 1336 | 1866 | -2820 | -1977 | -651 | -2572 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 130(T) | -1443 | -2096 | -3124 | -3287 | -4401 | -2292 | -3317 | -4200 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 131(G) | -2342 | -3617 | -1078 | -1138 | -4177 | 3145 | 602 | -3928 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 132(M) | -1412 | -1211 | -3721 | -3109 | -1195 | -3019 | -1922 | 1936 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 133(K) | -2382 | -3084 | -2779 | -1906 | -3480 | -3186 | -1412 | -563 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 134(L) | -2611 | -2332 | -4972 | -4508 | -1725 | -4314 | -3571 | -413 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 135(L) | -3561 | -3470 | -4972 | -5000 | -2527 | -4319 | -4274 | -2132 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 136(W) | -4178 | -3738 | -5370 | -5358 | -1338 | -4715 | -2806 | -2734 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 137(N) | 142 | -1989 | -2878 | -2702 | -3612 | 1917 | -2689 | -3274 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 138(T) | -3014 | -3363 | -4795 | -5109 | -5200 | -3551 | -4612 | -5219 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 139(A) | 2832 | -1972 | -4244 | -4339 | -4463 | -760 | -3712 | -4243 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 140(N) | -2239 | -3351 | -994 | -1271 | -4465 | -2566 | -2158 | -4418 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 141(M) | -1488 | 1901 | -3790 | -3168 | -1094 | -3058 | -1949 | -467 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 142(F) | -5308 | -4420 | -5610 | -5948 | 4547 | -4921 | -3048 | -4656 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 143(T) | -1333 | -1921 | -3518 | -3324 | -3642 | 123 | -3003 | -3307 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 144(H) | -2587 | -4410 | 687 | -793 | -4549 | -2586 | 4208 | -4506 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 145(P) | -777 | -3430 | -1097 | -93 | -4208 | -2659 | -1777 | -3899 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 146(R) | -550 | -1933 | -2864 | -2364 | -2830 | -927 | -2070 | -2310 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 147(F) | -4998 | -3935 | -5388 | -5739 | 3810 | -5262 | -1482 | -3879 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 148(M) | -1239 | -1212 | -2845 | -2242 | -1184 | -92 | -1512 | -579 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 149(H) | 593 | -3227 | 1964 | 442 | -3513 | -2264 | 3263 | -3299 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 150(G) | -4740 | -4445 | -5364 | -5738 | -6131 | 3840 | -5265 | -6903 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 151(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 152(A) | 3079 | -1981 | -4321 | -4534 | -4388 | 1735 | -3806 | -3957 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 153(A) | 2589 | -1888 | -3886 | -3806 | 2401 | -778 | -3205 | -3235 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 154(T) | -1493 | -2101 | -3952 | -4245 | -4536 | -2355 | -3794 | -4349 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 155(S) | 540 | -2055 | -3002 | -2865 | -4153 | -2245 | -2899 | -3915 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 156(C) | -1362 | 3283 | -2411 | -2080 | -3450 | -2276 | -2150 | -3137 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 157(D) | -1060 | -2512 | 2344 | 682 | 408 | -2001 | -688 | -2563 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 158(A) | 2203 | -1406 | -2316 | -1761 | 1179 | -2422 | -1413 | -1077 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 159(D) | -1108 | -2589 | 2572 | 1474 | -2902 | -2021 | -725 | -2655 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 160(V) | -2769 | -2275 | -5444 | -5141 | -2907 | -5216 | -5273 | 1475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 161(F) | -3052 | -2695 | -4546 | -4247 | 3203 | -4202 | -1494 | -2262 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 162(A) | 3042 | -2119 | -3426 | -3206 | -4202 | -2348 | -2904 | -3925 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 163(Y) | -3964 | -3336 | -5040 | -5039 | 1445 | -4787 | -1468 | -3083 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 164(A) | 3397 | -1996 | -4237 | -4525 | -4627 | -310 | -3879 | -4438 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 165(A) | 2613 | -1126 | -3505 | -2891 | -69 | -991 | -1733 | 690 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 166(A) | 2400 | -1805 | -2420 | -1934 | -2555 | 164 | -1841 | -2139 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 167(Q) | -3216 | -3916 | -3036 | -2340 | -4750 | -3581 | -1704 | -4191 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 168(V) | -1889 | -1609 | -4294 | -3746 | -1809 | -3673 | -2738 | 1337 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 169(K) | -1988 | -1815 | -3867 | -3265 | -61 | -3579 | -2411 | 608 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 170(H) | -256 | -2524 | -929 | 393 | -2858 | -2038 | 2453 | -2595 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 171(A) | 1760 | -2287 | -1318 | -859 | -3091 | 1259 | -1172 | -2823 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 172(M) | -3851 | -3263 | -6245 | -5660 | -1466 | -5999 | -4689 | 2110 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 173(D) | -2907 | -4984 | 3077 | 2640 | -5091 | -2646 | -2094 | -5089 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 174(I) | 1132 | -1695 | -4439 | -3899 | -1866 | -3834 | -2908 | 2259 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 175(T) | 2228 | -1974 | -4202 | -4278 | -4460 | 1638 | -3685 | -4242 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 176(K) | 692 | -1650 | -1775 | -1196 | -1722 | -2391 | 1742 | 714 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 177(E) | -1319 | -2706 | -1195 | 2572 | -3079 | -2247 | -809 | -2780 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 178(L) | -3875 | -3291 | -6254 | -5672 | -1457 | -5994 | -4687 | -834 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 179(G) | -2536 | -3114 | -2855 | -3225 | -5099 | 3685 | -3752 | -5315 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 180(A) | 2480 | -2007 | -3784 | -3834 | -4416 | 2311 | -3496 | -4197 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 181(E) | -2358 | -3954 | -688 | 3489 | -4290 | -2588 | -1658 | -4076 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 182(N) | -1197 | -1819 | -2024 | -1565 | -2415 | -764 | -1594 | -14 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 183(Y) | -4914 | -3888 | -5384 | -5705 | 1863 | -5254 | -1485 | -3696 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 184(V) | -3382 | -3135 | -5319 | -5407 | -3802 | -4379 | -4923 | -1179 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 185(F) | -245 | -1976 | -4569 | -3955 | 3611 | -3896 | -2734 | -630 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 186(W) | -6178 | -4896 | -5848 | -6204 | -4109 | -4952 | -4743 | -6650 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 187(G) | -3000 | -3383 | -4355 | -4713 | -5554 | 3708 | -4577 | -5789 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 188(G) | -4740 | -4445 | -5364 | -5738 | -6131 | 3840 | -5265 | -6903 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 189(R) | -5156 | -4675 | -5377 | -4998 | -5777 | -4641 | -4103 | -6271 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 190(E) | -4894 | -4877 | -3044 | 3932 | -5916 | -4252 | -4190 | -6541 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 191(G) | -4740 | -4445 | -5364 | -5738 | -6131 | 3840 | -5265 | -6903 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 192(Y) | 1128 | -2151 | -3615 | -3697 | -2169 | -2532 | -2750 | -3414 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 193(E) | -380 | -3053 | 283 | 3153 | -3334 | -2232 | -1107 | -3096 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 194(T) | -1103 | 1142 | -2635 | -2048 | 226 | -2529 | -1370 | -640 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 195(L) | -2451 | -3826 | 1344 | 380 | -3974 | -118 | -1886 | -3714 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 196(W) | 386 | -1134 | -2676 | -2101 | -1119 | -347 | 779 | -682 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 197(N) | 1013 | -2090 | -2983 | -3038 | -4320 | -2272 | -3125 | -4091 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 198(T) | -1798 | -2527 | -2472 | -2321 | -4196 | -165 | -2323 | -3864 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 199(D) | -2628 | -4493 | 3630 | -795 | -4689 | -2595 | -1891 | -4588 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 200(M) | -97 | -1073 | -3318 | -2701 | -998 | -2739 | -1606 | -472 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 201(K) | -294 | -2512 | -898 | 178 | -2843 | -95 | -659 | -2584 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 202(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 203(R) | 515 | -2100 | -67 | -484 | 1101 | -2050 | -726 | -1947 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 204(E) | 1191 | -2588 | -1433 | 3261 | -3616 | -2449 | -2074 | -2773 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 205(L) | -1513 | -1884 | -2260 | -1562 | -1940 | -2676 | -1221 | -1499 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 206(D) | -745 | -4362 | 3177 | 2316 | -4570 | -2552 | -1843 | -4464 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 207(H) | -1159 | -2582 | -194 | -438 | -2895 | -2097 | 3162 | -2638 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 208(M) | -667 | -1277 | -3818 | -3186 | 164 | -3048 | -1914 | 1188 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 209(A) | 2605 | -2502 | -1660 | -1121 | -3271 | 74 | -1239 | -2944 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 210(R) | -254 | -2474 | -853 | 1591 | -2798 | -1977 | -630 | -2545 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 211(F) | 948 | -1050 | -3245 | -2644 | 3385 | -798 | -1571 | 28 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 212(F) | -2629 | -2312 | -4914 | -4321 | 2717 | -4278 | -2852 | -858 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 213(H) | -1020 | -2488 | 1097 | -309 | -2812 | -1983 | 2931 | -2560 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 214(M) | -359 | -1579 | -4116 | -3488 | 569 | -3396 | -2253 | 143 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 215(A) | 2548 | 947 | -3783 | -3180 | -1231 | -3043 | -2001 | -310 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 216(V) | 787 | 844 | -1857 | -1289 | -1397 | -389 | 570 | 555 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 217(D) | -208 | -3213 | 2902 | 1429 | -3501 | -609 | -1166 | -3288 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 218(Y) | -4881 | -3957 | -5031 | -5318 | -128 | -5096 | 2506 | -3985 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 219(A) | 2495 | -1569 | -1878 | -1335 | -1756 | -621 | -1246 | -153 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 220(K) | -986 | -2457 | -27 | 762 | -2778 | -1959 | 1449 | -834 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 221(E) | -254 | -2564 | 1369 | 2076 | -2882 | -2009 | -700 | -2635 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 222(I) | -2688 | -3306 | -3275 | -2169 | -3815 | -3397 | -1438 | 3178 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 223(G) | -2637 | -3974 | 390 | -1153 | -5020 | 3488 | -2357 | -5035 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 224(Y) | -4400 | -3612 | -5188 | -5335 | 3494 | -5009 | 1099 | -3457 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 225(D) | -991 | -2464 | 1721 | 213 | -2784 | 477 | 823 | -2534 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 226(G) | 624 | -1134 | -2638 | -2059 | -1134 | 2287 | -1399 | -199 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 227(Q) | -1134 | -2497 | -1028 | -462 | -2811 | -2101 | -720 | -622 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 228(F) | -3742 | -3197 | -6068 | -5484 | 3900 | -5732 | -4235 | 207 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 229(L) | 1551 | -2659 | -4811 | -4712 | -2094 | -3608 | -3887 | -1436 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 230(I) | -3359 | -2829 | -5847 | -5421 | -1855 | -5595 | -4779 | 3551 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 231(E) | -4894 | -4877 | -3044 | [redacted] | -5916 | -4252 | -4190 | -6541 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 232(P) | -5161 | -4627 | -5479 | -5844 | -6102 | -4639 | -5314 | -6971 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 233(K) | -4797 | -4587 | -4692 | -4321 | -5682 | -4474 | -3615 | -5879 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 234(P) | -513 | -2636 | -4047 | -4375 | -4929 | -2842 | -4112 | -4853 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 235(K) | -1854 | -3064 | -1724 | -1121 | -3517 | -2674 | -1100 | -3136 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 236(E) | -4894 | -4877 | -3044 | 3932 | -5916 | -4252 | -4190 | -6541 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 237(P) | -5161 | -4627 | -5479 | -5844 | -6102 | -4639 | -5314 | -6971 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 238(T) | -1947 | -2421 | -3165 | -2413 | -2978 | -2880 | -1827 | -2467 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 239(K) | -484 | -2389 | -1045 | -493 | -2703 | 1249 | -755 | -2401 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 240(H) | -3186 | -4015 | 2118 | -1712 | 238 | -3267 | 4734 | -4101 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 241(Q) | -2896 | -3052 | -3270 | -3166 | -2696 | -3713 | -2865 | 2002 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 242(Y) | -4742 | -3801 | -5383 | -5639 | 926 | -5232 | -1545 | -3295 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 243(D) | -3103 | -3191 | 3532 | -2696 | 707 | -3852 | -2334 | -1817 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 244(F) | -78 | -1005 | -3333 | -2714 | 2786 | -2679 | -1509 | -502 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 245(D) | -2674 | -3777 | 3647 | -1466 | -4960 | -2802 | -2593 | -4914 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 246(V) | 1871 | -1480 | -3615 | -3153 | -1890 | -2627 | -2297 | -306 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 247(A) | 2611 | -2786 | -991 | 446 | -3556 | 1526 | -1508 | -3306 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 248(T) | -1575 | -2397 | -1716 | -1668 | -3555 | -2353 | 2795 | -3355 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 249(A) | 1906 | -1213 | -2611 | -2044 | -1274 | -749 | -1459 | -806 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 250(I) | -1289 | -1106 | -3622 | -2990 | 447 | -2843 | 556 | 2280 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 251(A) | 2994 | -2060 | -3318 | -3466 | -4480 | 1477 | -3420 | -4277 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 252(F) | -4510 | -3775 | -5456 | -5636 | 4401 | -5131 | -2010 | -2624 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 253(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 254(L) | -3584 | -3041 | -6020 | -5465 | -1547 | -5724 | -4559 | 2056 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 255(Q) | -1021 | 248 | 260 | 920 | -2812 | -1989 | 1097 | -2558 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 256(Q) | -387 | -2464 | -846 | 769 | -2785 | -1226 | -626 | -2533 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 257(Y) | -4615 | -3726 | -5293 | -5520 | 1958 | -5121 | 2666 | -3552 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 258(G) | -747 | -4382 | 2188 | 1513 | -4595 | 2379 | -1856 | -4494 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 259(L) | -2849 | -3066 | -3826 | -2761 | -2395 | -3664 | 902 | -2487 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 260(D) | -1081 | -2552 | 2320 | 1262 | -2865 | -682 | -702 | -2615 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 261(K) | -1509 | -3060 | 1864 | 1703 | -3356 | -449 | -1058 | -3134 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 262(H) | -1002 | -2068 | 1504 | 952 | -2245 | -2050 | 1617 | -504 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 263(F) | -2939 | -2542 | -4828 | -4519 | 3955 | -4337 | -1860 | 649 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 264(K) | -3285 | -3787 | -3212 | -3069 | -5220 | 1761 | -2834 | -5042 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 265(L) | -3149 | -2690 | -5597 | -5054 | 1255 | -5177 | -4100 | 997 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 266(N) | -4313 | -4359 | -3728 | -4093 | -5358 | -4169 | -4424 | -6344 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 267(I) | -2513 | -2147 | -4957 | -4435 | -1823 | -4442 | -3529 | 2351 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 268(E) | -4894 | -4877 | -3044 | 3932 | -5916 | -4252 | -4190 | -6541 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 269(A) | 2088 | 1365 | -3378 | -2828 | 487 | 698 | -1799 | -836 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 270(N) | 11 | -2335 | -2305 | -2622 | -4638 | 1373 | -3163 | -4488 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 271(H) | -5506 | -4764 | -5029 | -5331 | -4362 | -4732 | 5444 | -6634 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 272(A) | 3116 | -3101 | -1551 | 1884 | -4671 | -2707 | -2717 | -4449 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 273(T) | -1255 | -2072 | -1391 | -875 | -1774 | -2311 | -946 | -1843 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 274(M) | -4049 | -3435 | -6360 | -5790 | -1437 | -6127 | -4754 | -942 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 275(A) | 3290 | -2018 | -4238 | -4530 | -4608 | -2288 | -3883 | -4417 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 276(G) | -2006 | -2603 | -2686 | -2983 | -51 | 3566 | -2982 | -4201 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 277(H) | -3898 | -3709 | -4252 | -4240 | -1538 | -4376 | 4892 | -2923 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 278(T) | -1794 | -2944 | 99 | -1062 | -3953 | -2361 | -1803 | -3744 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 279(F) | -4666 | 1469 | -5329 | -5600 | 4144 | -5120 | -1487 | -3637 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 280(E) | -993 | -2363 | -876 | 2071 | -2644 | -1981 | 2018 | -2365 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 281(H) | -3635 | -4141 | -2481 | -2626 | -2997 | -3692 | 5257 | -4886 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 282(E) | -310 | -4317 | 520 | 3226 | -4661 | 1262 | -1926 | -4565 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 283(I) | -3109 | -2594 | -5687 | -5258 | -2040 | -5447 | -4789 | 2687 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 284(R) | 1930 | -2801 | -1080 | 241 | -3166 | -2275 | -912 | -2874 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 285(Q) | -1119 | 867 | -3256 | -2635 | 296 | -2643 | -1503 | -469 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 286(A) | 3647 | -2999 | -4758 | -5092 | -5144 | -3219 | -4514 | -5091 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 287(R) | -438 | -1218 | -2385 | -1792 | -1208 | -2507 | -1291 | 204 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 288(W) | 180 | -1750 | 1481 | 940 | -1841 | -2159 | -866 | 977 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 289(H) | 1869 | -1372 | -1935 | -1371 | 128 | -2386 | 2326 | -951 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 290(G) | -2602 | -4486 | 1035 | 201 | -4670 | 3031 | -1891 | -4579 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 291(M) | 251 | -1162 | -3387 | -2773 | -1077 | -2851 | -1716 | 431 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 292(L) | -4115 | -3482 | -6205 | -5777 | 1065 | -5951 | -3943 | -1092 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 293(G) | -2777 | -2893 | -4388 | -4459 | 2368 | 3236 | -2287 | -2772 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 294(H) | -1953 | -2855 | -1413 | -1605 | -3687 | -2513 | 3228 | -4087 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 295(I) | -2891 | -2384 | -5528 | -5155 | -2443 | -5319 | -4964 | 3155 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 296(D) | -4886 | -4905 | 4186 | -3339 | -5949 | -4221 | -4195 | -6666 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 297(A) | 3106 | -2388 | -4425 | -4439 | -2714 | -3005 | -3693 | -1978 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 298(N) | -4313 | -4359 | -3728 | -4093 | -5358 | -4169 | -4424 | -6344 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 299(Q) | -1050 | -2442 | -224 | -369 | -2747 | 1053 | -681 | -2473 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 300(G) | -2973 | -3542 | -2659 | -2952 | -5121 | 3336 | -3467 | -5361 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 301(D) | -1715 | -3268 | 3029 | -581 | -3564 | -2306 | 1979 | -3347 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 302(M) | -558 | -1400 | -1835 | -1269 | -1420 | 890 | -1097 | -990 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 303(Q) | -1128 | -1286 | -2178 | -1630 | -1284 | -2459 | -1259 | 1117 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 304(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 305(L) | -1103 | -1400 | -1916 | -1340 | -1417 | -2382 | -1126 | 637 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 306(G) | -4196 | -3631 | -4960 | -5174 | 1957 | 3226 | -1563 | -3645 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 307(W) | -4933 | -4706 | 2215 | -3821 | -3602 | -4386 | -3866 | -6001 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 308(D) | -3069 | -4812 | 3638 | -1083 | -5072 | -2828 | -2301 | -5194 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 309(T) | -1461 | -1742 | 1783 | -1718 | -1846 | -2672 | -1664 | 872 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 310(D) | -3546 | -4222 | 3684 | -2153 | -3783 | -3505 | -3081 | -3676 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 311(E) | -1512 | -2828 | -1244 | 2417 | -3192 | -2375 | -959 | -2865 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 312(F) | -4608 | -3778 | -5235 | -5513 | 4303 | -5053 | -1486 | -3725 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -61 | -8312 | -4703 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 313(P) | -1888 | -2446 | -3444 | -3643 | -4185 | 1827 | -3530 | -4064 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8258 | -9300 | -894 | -1115 | -545 | -1668 | * |
| | | | | | | | | |
| 314(T) | -273 | -2001 | -1163 | -623 | -2217 | -2089 | 1932 | 12 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 315(D) | -1847 | -3447 | 2925 | 425 | -3721 | 1133 | -1341 | -3520 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 316(V) | -1151 | -1254 | 1271 | -1753 | -1247 | -2524 | -1330 | 1068 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 317(Y) | -1131 | -1223 | -2356 | 151 | -1178 | -2505 | -1285 | 128 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 318(E) | -66 | -2443 | 1230 | 1971 | -2759 | -1957 | -616 | -2506 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 319(T) | 1585 | -1443 | -1997 | -1450 | -1562 | -2339 | -1262 | -37 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 320(T) | 1918 | -1866 | -4124 | -4033 | -3557 | -2298 | -3314 | -2998 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 321(F) | -55 | -1078 | -2947 | -2361 | 2116 | -724 | -1483 | -590 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 322(A) | 2342 | -969 | -3431 | -2800 | 739 | -2681 | -1551 | 746 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 323(M) | -2508 | -2232 | -4761 | -4150 | -1237 | -4143 | -2920 | -798 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 324(Y) | -1183 | -1021 | -3411 | -2790 | -854 | -2712 | 739 | -517 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 325(E) | -1745 | -3328 | 1991 | 2779 | -3608 | -2301 | -1256 | -3398 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 326(I) | -2844 | -2383 | -5395 | -4937 | 151 | -5038 | -4287 | 2475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 327(L) | -3363 | -2830 | -5881 | -5398 | -1770 | -5628 | -4722 | 1688 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 328(K) | -365 | -2488 | 41 | 1718 | -2813 | -1989 | -641 | -2559 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 329(N) | 716 | -2390 | -858 | 192 | -377 | -873 | 712 | -2414 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 330(G) | 66 | -2442 | 172 | -2179 | -4601 | 3363 | -2899 | -4434 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -244 | -8312 | -2711 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 331(G) | -1329 | -1980 | -1953 | -1910 | 214 | 3238 | -2065 | -2824 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8076 | -9118 | -894 | -1115 | -1192 | -831 | * |
| | | | | | | | | |
| 332(F) | -2519 | -2199 | -4819 | -4218 | 2372 | -4220 | -2969 | 1473 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -1585 | -8137 | -593 | -894 | -1115 | -1458 | -653 | * |
| | | | | | | | | |
| 333(D) | -901 | -2417 | 2169 | 499 | -2834 | 1682 | -424 | -2665 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -555 | -6574 | -1696 | -894 | -1115 | -3087 | -181 | * |
| | | | | | | | | |
| 334(G) | -771 | -1114 | -1327 | -1503 | -2474 | 3336 | -1576 | -2519 |
| - | -148 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -1217 | -830 | -7094 | -156 | -3286 | -3231 | -162 | * |
| | | | | | | | | |
| 335(P) | -947 | -1231 | -1366 | -1466 | -2201 | -1324 | -1470 | -2177 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -33 | -6052 | -7094 | -894 | -1115 | -2145 | -370 | * |
| | | | | | | | | |
| 336(G) | 1354 | -1173 | -535 | -238 | -2069 | 1779 | -486 | -1725 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -21 | -6683 | -7725 | -894 | -1115 | -113 | -3725 | * |
| | | | | | | | | |
| 337(Y) | 923 | -2098 | -152 | -490 | -2292 | 773 | -737 | -1954 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 338(T) | -596 | -2454 | -27 | 443 | -2775 | -1955 | 675 | -2525 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 339(G) | -4740 | -4445 | -5364 | -5738 | -6131 | 3840 | -5265 | -6903 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 340(G) | -2931 | -3331 | -4304 | -4658 | -5520 | 3378 | -4536 | -5732 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 341(L) | -1644 | -1468 | -3782 | -3157 | 438 | -3201 | -2041 | 710 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 342(N) | -3133 | -4000 | -1625 | -1938 | -3244 | -3217 | 3075 | -4941 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 343(F) | -5308 | -4420 | -5610 | -5948 | 4547 | -4921 | -3048 | -4656 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 344(D) | -3598 | -4594 | 4015 | -1825 | -3014 | -3354 | -2628 | -5167 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 345(A) | 3015 | -2000 | -3678 | -3337 | 863 | -3026 | -1786 | -1924 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 346(K) | -3437 | -3998 | -3427 | -2630 | -4447 | -3747 | 1328 | -4350 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 347(V) | 467 | -1371 | -3451 | -2935 | -1595 | -2710 | -2060 | -662 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 348(R) | -1896 | -2633 | -2566 | -1895 | -3409 | -2759 | -1580 | -3013 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 349(R) | -5156 | -4675 | -5377 | -4998 | -5777 | -4641 | -4103 | -6271 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 350(T) | 361 | -2459 | -832 | 770 | -2780 | -972 | -644 | -2526 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 351(S) | -2650 | -4418 | 471 | 1677 | -4792 | -2597 | -2009 | -4725 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -383 | -8312 | -2119 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 352(F) | -1686 | -1643 | -3313 | -2900 | 3471 | -2934 | -1275 | -1319 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -9 | -7937 | -8980 | -894 | -1115 | -254 | -2629 | * |
| | | | | | | | | |
| 353(D) | 395 | -2586 | 2498 | 794 | -2902 | -678 | -718 | -2657 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 354(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 355(P) | 574 | -1802 | 174 | 209 | 242 | -2139 | -841 | -414 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 356(E) | -1666 | -3205 | 2244 | 2653 | -3457 | -2276 | -1194 | -3250 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 357(D) | -3359 | -4413 | 3698 | -1801 | -5459 | 1768 | -2962 | -5658 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 358(L) | -3209 | -2692 | -5753 | -5295 | -1892 | -5491 | -4700 | 887 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 359(W) | -3018 | -2633 | -4761 | -4491 | 3489 | -4266 | -1661 | 1631 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 360(Y) | 828 | -1415 | -340 | -305 | -1437 | -2319 | 1172 | 1116 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 361(A) | 2490 | -1883 | -3530 | -3268 | 392 | 1317 | -2915 | -3137 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 362(H) | 1671 | -2732 | -3104 | -3318 | -3618 | -2860 | 4890 | -4439 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 363(I) | 372 | -1510 | -2804 | -2153 | -1581 | -2814 | -1570 | 3136 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 364(A) | 2824 | -1953 | -4254 | -4336 | -4278 | 1795 | -3663 | -3992 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 365(G) | 440 | 2735 | -4017 | -4164 | -4414 | 3094 | -3664 | -4182 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 366(M) | -3435 | -3070 | -5645 | -5292 | -1686 | -5048 | -4285 | 177 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 367(D) | -2500 | -3942 | 3572 | -1015 | -4586 | -2633 | -1974 | -4469 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 368(T) | 1100 | -1830 | -2203 | -1732 | -2569 | -2230 | -1735 | -2187 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 369(Y) | -4358 | -3577 | -5299 | -5430 | 3019 | -5078 | -1573 | -2961 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 370(A) | 3106 | -2388 | -4425 | -4439 | -2714 | -3005 | -3693 | -1978 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 371(R) | 722 | -1126 | -2900 | -2287 | -1095 | -2649 | -1470 | 1035 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 372(G) | 1248 | -1889 | -3801 | -3776 | -3523 | 2775 | -3223 | -3143 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 373(L) | -2143 | -2281 | -3312 | -2427 | 697 | -3298 | -1645 | -1581 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 374(K) | -1243 | -2663 | -484 | 1864 | -3023 | -2175 | -770 | -2741 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 375(V) | 135 | -1595 | -1634 | -1081 | -1703 | -685 | -1060 | -548 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 376(A) | 3481 | -2081 | -4434 | -4657 | -4120 | -2450 | -3905 | -2895 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 377(A) | 1962 | -2221 | -948 | 716 | -2449 | -2010 | 1143 | -2137 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 378(K) | 2235 | -3511 | -3265 | -2182 | -4396 | -3362 | -1483 | -3787 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 379(M) | -3847 | -3277 | -6201 | -5593 | 2394 | -5898 | -4497 | 240 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 380(I) | -1275 | -1329 | -2647 | -1990 | -1321 | -2643 | 565 | 2209 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 381(E) | 944 | -2571 | 656 | 2380 | -2887 | -2009 | -705 | -2642 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 382(D) | -598 | -4837 | 3833 | 578 | -5072 | -2648 | -2116 | -5055 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 383(P) | -1003 | -2474 | -144 | -294 | -2795 | 1002 | -631 | -2545 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 384(F) | 81 | -1956 | -1130 | 1500 | 1552 | -2090 | -781 | -1736 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 385(F) | -2039 | -1782 | -4382 | -3777 | 2108 | -3679 | -2463 | 807 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 386(D) | -987 | 436 | 2031 | 1591 | -2780 | -1958 | -618 | -2531 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 387(E) | -507 | -2601 | 840 | 1955 | -2917 | -2021 | -726 | -2673 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 388(M) | 1019 | -961 | -3469 | -2834 | 1400 | -2682 | -1552 | 1329 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 389(M) | -1149 | -1013 | -3253 | -2628 | -962 | -2671 | -1525 | 2021 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 390(A) | 1814 | -2486 | 567 | 1223 | -2807 | -1975 | -640 | -2557 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 391(E) | 1007 | -2465 | 333 | 2274 | -2786 | -1961 | 536 | -2537 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 392(R) | 73 | -2800 | -1762 | -1095 | -3205 | -2591 | -1056 | -2829 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 393(Y) | -2721 | -3376 | -2973 | -2154 | -3188 | -727 | -1495 | -3439 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 394(A) | 1285 | -2377 | -872 | 585 | -2664 | -1977 | -638 | -2390 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 395(S) | 131 | -2423 | 336 | 386 | -341 | -50 | -762 | -2464 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 396(F) | -3512 | -3303 | 10 | 1365 | 3217 | -4329 | -1411 | -3102 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 397(D) | -990 | -2441 | 1836 | -293 | -2754 | -1964 | -625 | -2498 |
| - | -147 | -500 | 233 | 43 | -381 | 399 | 105 | -627 |
| - | -466 | -1865 | -9354 | -73 | -4335 | -701 | -1378 | * |
| | | | | | | | | |
| 398(E) | -84 | -2457 | -258 | 1626 | -2777 | -455 | -617 | -2527 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 399(G) | -1414 | -2476 | -1090 | 1358 | -2997 | 2543 | -1296 | -2678 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 400(I) | 128 | -1033 | -3266 | -2651 | -1007 | -2701 | -1569 | 2545 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 401(G) | 129 | -2062 | -3816 | -3956 | -3724 | 3250 | -3484 | -3244 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -79 | -8312 | -4317 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 402(Q) | 1462 | -2384 | -811 | 38 | -2692 | -1184 | -584 | -2431 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8240 | -9282 | -894 | -1115 | -509 | -1750 | * |
| | | | | | | | | |
| 403(D) | 286 | -2477 | 2128 | 1000 | -2797 | -1001 | -633 | -2548 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 404(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 405(I) | -1225 | -1056 | -3499 | -2876 | 1632 | 939 | -1661 | 2615 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -183 | -8312 | -3109 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 406(E) | -156 | -1264 | -2093 | 2168 | -1299 | -2445 | -1234 | 765 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8137 | -9179 | -894 | -1115 | -1458 | -653 | * |
| | | | | | | | | |
| 407(E) | 360 | -2380 | 448 | 1932 | -2699 | -673 | -533 | -2451 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -8 | -8137 | -9179 | -894 | -1115 | -373 | -2136 | * |
| | | | | | | | | |
| 408(G) | -306 | -1754 | -2167 | -1874 | -1807 | 2904 | -1682 | -1676 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 409(K) | 356 | -2456 | -833 | 230 | -2777 | -1959 | 716 | -2527 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 410(A) | 1615 | -2218 | 495 | 834 | -2445 | -2009 | 454 | -2132 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 411(D) | -1190 | -2605 | 1765 | 410 | -2890 | -2073 | -813 | -2627 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 412(F) | -2934 | -2588 | -5133 | -4524 | 2606 | -4635 | -3270 | -872 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 413(E) | 1449 | -2456 | -323 | 1515 | -2776 | -1957 | 1150 | -2526 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 414(D) | -185 | -2555 | 2037 | 1092 | -2872 | -2002 | -694 | -2626 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 415(L) | -76 | -1139 | -2558 | -1967 | -1115 | -2532 | -1344 | -133 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 416(E) | 1511 | 1129 | -1595 | 1751 | -1576 | -2259 | -999 | -1157 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 417(K) | 1530 | -2471 | -17 | 1044 | -2791 | -1963 | -628 | -2542 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 418(Y) | -3642 | -3323 | -4353 | -3980 | 2403 | -4454 | 1728 | -3120 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 419(A) | 2435 | -1847 | -629 | 808 | -1986 | -901 | -1033 | 1094 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 420(E) | 67 | -2239 | 224 | 1346 | -452 | -709 | -673 | 230 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 421(D) | -160 | -2437 | 1202 | 1064 | 1123 | -1958 | -618 | -2495 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 422(H) | -986 | -2410 | 1343 | -303 | -2711 | -1966 | 2413 | -86 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -72 | -8312 | -4460 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 423(N) | 209 | -2403 | -80 | 227 | -2718 | 589 | 404 | -2465 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -110 | -8247 | -3828 | -894 | -1115 | -1040 | -961 | * |
| | | | | | | | | |
| 424(Q) | -887 | -2330 | 1492 | 1492 | -2638 | -585 | 686 | -431 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -848 | -8144 | -1181 | -894 | -1115 | -1434 | -667 | * |
| | | | | | | | | |
| 425(A) | 2076 | -2279 | 957 | 892 | -2676 | -1567 | -534 | -2428 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -942 | -7310 | -1080 | -894 | -1115 | -2709 | -239 | * |
| | | | | | | | | |
| 426(E) | -381 | -1604 | 25 | 1852 | -1759 | -1234 | 69 | -1535 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -246 | -6394 | -2788 | -894 | -1115 | -3145 | -173 | * |
| | | | | | | | | |
| 427(L) | -1340 | -1019 | -3303 | -2788 | -110 | -3122 | -2028 | 1936 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -30 | -6178 | -7220 | -894 | -1115 | -1475 | -643 | * |
| | | | | | | | | |
| 428(A) | 996 | -971 | -924 | 841 | -1028 | -1637 | -368 | 796 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -15 | -7202 | -8244 | -894 | -1115 | -137 | -3462 | * |
| | | | | | | | | |
| 429(I) | 1151 | -1159 | -2438 | -631 | 77 | -2505 | -1313 | 1901 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 430(Q) | 1025 | -2430 | -838 | 1164 | -2741 | -144 | -619 | -1061 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 431(N) | 351 | -2447 | 158 | 275 | -2764 | -782 | 1793 | -2512 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 432(K) | -983 | -2449 | 67 | 142 | -2768 | -1958 | 1110 | -2516 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 433(S) | -1458 | -2150 | -2472 | -2476 | -3880 | 1432 | -2696 | -3646 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 434(G) | -1089 | 904 | 851 | -1707 | 768 | 2205 | -1253 | -740 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 435(H) | 818 | -2554 | -999 | -433 | -2894 | -123 | 2729 | -2621 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 436(Q) | -344 | -1587 | -3692 | -3091 | 1883 | -3276 | -2091 | 243 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 437(E) | -2248 | -2420 | -2316 | 3407 | -2671 | -3394 | -2628 | 379 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 438(R) | 204 | -2356 | -880 | 414 | -2634 | -1980 | 1194 | -2355 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 439(L) | -358 | -1562 | -4144 | -3535 | -1264 | -3440 | -2330 | 1655 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 440(K) | -1751 | -3345 | 1209 | 2128 | -3630 | -2309 | -1249 | -3421 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 441(Q) | 854 | -2432 | 264 | -308 | -2743 | -1971 | -641 | -2483 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 442(L) | -1684 | -1481 | -3911 | -3303 | -1234 | -3289 | -2179 | 1310 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 443(I) | 1388 | -1660 | -4350 | -3774 | 339 | -3691 | -2667 | 1898 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 444(N) | -429 | -1301 | -2255 | -1692 | -1310 | -2510 | -1317 | 965 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 445(H) | -989 | -2462 | 1140 | 1287 | -2782 | -1959 | 2073 | -2533 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 446(Y) | -570 | -3515 | -4109 | -4216 | -224 | -4378 | 3141 | -3593 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -7 | -8312 | -9354 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 447(L) | -2249 | 853 | -4575 | -3952 | -1251 | -3905 | -2751 | 1774 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -183 | -8312 | -3108 | -894 | -1115 | -701 | -1378 | * |
| | | | | | | | | |
| 448(F) | -1766 | -1521 | -4129 | -3513 | 1880 | -3413 | -2296 | 1109 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -152 | -8137 | -3370 | -894 | -1115 | -1459 | -652 | * |
| | | | | | | | | |
| 449(G) | -169 | -2301 | 1066 | 908 | -2620 | 1545 | -453 | -2372 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -149 | -7993 | -3412 | -894 | -1115 | -1838 | -473 | * |
| | | | | | | | | |
| 450(A) | 1384 | -1474 | -1057 | 562 | -1568 | -1895 | -607 | -94 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -253 | -7853 | -2678 | -894 | -1115 | -2104 | -382 | * |
| | | | | | | | | |
| 451(R) | -58 | 1062 | -1873 | -1289 | -813 | -2052 | -847 | 394 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | -368 | -7612 | -2186 | -894 | -1115 | -2433 | -295 | * |
| | | | | | | | | |
| 452(G) | 887 | -1428 | -559 | -11 | -1592 | 1139 | -219 | -1235 |
| - | -149 | -500 | 233 | 43 | -381 | 399 | 106 | -626 |
| - | -296 | -7258 | -2484 | -894 | -1115 | -2747 | -233 | * |
| | | | | | | | | |
| 453(K) | 359 | -1852 | -165 | 1529 | -2167 | -1348 | -26 | -1905 |
| - | -147 | -500 | 233 | 43 | -381 | 398 | 105 | -626 |
| - | -1078 | -936 | -8022 | -95 | -3976 | -125 | -3592 | * |
| | | | | | | | | |
| 454(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | -148 | -500 | 233 | 43 | -381 | 399 | 107 | -627 |
| - | -166 | -3224 | -9354 | -1658 | -550 | -701 | -1378 | * |
| | | | | | | | | |
| 455(W) | -204 | 887 | 46 | 246 | -204 | -619 | 891 | -475 |
| - | * | * | * | * | * | * | * | * |
| - | * | * | * | * | * | * | * | * |

| K | L | M | N | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|
| m->e | | | | | | | | |
| 338 | -941 | 659 | 291 | -334 | 598 | 147 | -143 | 7 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -3750 | 38 | -736 | -3643 | -3893 | -3438 | -3659 | -3185 | -1974 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -145 | -2417 | -1502 | 888 | 1044 | 650 | -666 | 634 | -860 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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|---|---|---|---|---|---|---|---|---|
| * | | | | | | | | |
| 2070 | -2275 | -1362 | -444 | 1490 | 1183 | 432 | 193 | 169 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -721 | 275 | 394 | 46 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 338 | -941 | 659 | 291 | -334 | 598 | 147 | -143 | 7 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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|---|---|---|---|---|---|---|---|---|
| * | | | | | | | | |
| 519 | 2067 | 848 | -1624 | -2718 | 1781 | 725 | -1719 | -1418 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -2791 | 1720 | 3132 | -2694 | -3054 | -2373 | -2581 | -2141 | -1411 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 508 | -2930 | -2076 | -1300 | -2572 | -823 | 1599 | -500 | -1463 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 1166 | -2488 | -1564 | 387 | -2070 | 471 | 2128 | -309 | 332 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 939 | -1605 | -891 | -1427 | -2466 | -1072 | -1499 | -411 | -1115 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 1262 | -3125 | -2452 | -2120 | -3365 | 1862 | 561 | -2654 | -2443 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -2776 | -4946 | -4322 | 291 | -3293 | -2075 | -3657 | -2438 | -2858 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 101 | -2489 | -1598 | 278 | -1086 | 2837 | 1581 | -1029 | 1381 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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|---|---|---|---|---|---|---|---|---|
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| -4583 | -6183 | -5913 | -3747 | -4767 | -4175 | -4884 | -4792 | -5049 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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|---|---|---|---|---|---|---|---|---|
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -3734 | -780 | -3561 | -3019 | 3618 | -3530 | -3731 | -2122 | -2300 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -5234 | 2786 | -540 | -5318 | -5008 | -4398 | -4954 | -4964 | -3282 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 118 |

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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 123 | -2065 | -1235 | -765 | 927 | -341 | -870 | -977 | 771 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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|---|---|---|---|---|---|---|---|---|
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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|---|---|---|---|---|---|---|---|---|
| * | | | | | | | | |
| 2247 | -1301 | -1757 | -811 | -2263 | 491 | 1587 | -1121 | -1167 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 625 | 584 | -515 | -1883 | -2692 | -1414 | 1794 | -1698 | -1203 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 41 | -1894 | -1096 | -841 | 683 | 68 | -936 | -136 | -956 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 1161 | 389 | -1533 | 1125 | -131 | -168 | 2 | -164 | 557 |
| 211 | -466 | -721 | 276 | 393 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 1433 | -354 | 117 | 801 | -2020 | 1472 | 466 | -836 | -887 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 308 | -2493 | -1568 | -601 | 199 | 1422 | -727 | 187 | -166 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 338 | -941 | 659 | 291 | -334 | 598 | 147 | -143 | 7 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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|---|---|---|---|---|---|---|---|---|
| * | | | | | | | | |
| 1493 | -2471 | -1545 | 270 | -1061 | -157 | -422 | -422 | 54 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 1572 | -2487 | -1561 | 676 | -2060 | 1100 | -720 | -173 | -45 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -855 | -2839 | -2606 | -3260 | -4404 | -2976 | 133 | -3614 | -3524 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -847 | -1803 | -1059 | -1095 | -2363 | -746 | -1268 | -1239 | -1109 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 878 | -2442 | -1522 | 203 | -2052 | 1476 | 325 | -866 | -41 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 1671 | -360 | -1539 | -594 | -622 | 1599 | 1436 | -865 | 334 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| 610 | 446 | -1456 | -633 | -2072 | 1010 | 1766 | -263 | -928 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |

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| * | | | | | | | | |
| -3166 | 2331 | -351 | -3080 | -3407 | -2740 | -2969 | -2549 | -1736 |
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| * | | | | | | | | |
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| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -3436 | 1384 | -620 | -3337 | -3648 | -3052 | -3269 | -2821 | -1888 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -202 | -2477 | 1021 | 761 | -2054 | 1813 | 414 | 304 | -929 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -3871 | -3148 | -3048 | -3397 | -4538 | -3440 | -3721 | -3699 | -3833 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -3586 | 2535 | 1585 | -3549 | -3766 | -365 | -3344 | -3026 | -2171 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
| -3142 | 1880 | 1149 | -3061 | -3355 | -2682 | -2926 | -2524 | -64 |
| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
| * | | | | | | | | |
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| 210 | -466 | -720 | 275 | 394 | 45 | 96 | 359 | 117 |
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[1]Program name and version
[2]Name of the input sequence alignment file
[3]Length of the alignment: include indels
[4]Type of residues
[5]Map of the match states to the columns of the alignment
[6]Commands used to generate the file: this one means that hmmbuild (default parameters) was applied to the alignment file
[7]Commands used to generate the file: this one means that hmmcalibrate (default parameters) was applied to the hmm profile
[8]Number of sequences in the alignment
[9]When the file was generated
[10]The trasition probability distribution for the null model (single G state).
[11]The symbol emission probability distribution for the null model (G state); consists of K integers. The null probability used to convert these back to model probabilities is 1/K.
[12]The extreme value distribution parameters μ and lambda respectively, both floating point values. Lambda is positive and nonzero. These values are set when the model is calibrated with hmmcalibrate.

**The claims defining the invention are as follows:**

1. A recombinant bacterial strain selected from the group consisting of *Zymomonas* and *Zymobacter* comprising a chimeric gene, introduced by transformation, the chimeric gene comprising:
a) an isolated nucleic acid molecule comprising a *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter that has a base substitution at position 116; wherein the position number is of SEQ ID NO:1; which is an improved P*gap*; and
b) an operably linked isolated nucleic acid molecule encoding a xylose isomerase enzyme.

2. The recombinant strain of claim 1, wherein the base substitution is a T replacing G.

3. The recombinant strain of claim 2 wherein the improved P*gap* comprises a sequence selected from the group consisting of SEQ ID NO: 4, 6, 7, 9, 10, and 12.

4. The recombinant strain of claim 1, additionally transformed with genes for expression of xylulokinase, transaldolase and transketolase.

5. The recombinant strain of claim 1, wherein the chimeric gene further comprises an operably linked isolated nucleic acid molecule encoding xylulokinase, forming an operon.

6. The recombinant strain of claim 1, wherein the xylose isomerase enzyme is a protein having an E-value parameter of less than or equal to $3x10^{-10}$ when queried using the Pfam Profile HMM for the xylA family of proteins given in Table 3 and having the four catalytic site residues: histine 54, aspartic acid 57, glutamic

acid 181, and lysine 183, with the position numbers in reference to the *Streptomyces albus* xylose isomerase sequence (SEQ ID NO:84).

7. The recombinant strain of claim 1, wherein the xylose isomerase enzyme has an amino acid sequence that has 90% amino acid identity to a sequence selected from the group consisting of SEQ ID NOs; 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, and 104.

8. A process for engineering a bacterial strain selected from the group consisting of *Zymomonas* and *Zymobacter* comprising:
transforming with a chimeric gene comprising:

a) an isolated nucleic acid molecule comprising a *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter that has a base substitution in a position selected from the group consisting of position 116, position 217, and both position 116 and 217; wherein the position numbers are of SEQ ID NO:1; which is an improved P*gap*; and

b) an operably linked isolated nucleic acid molecule encoding a xylose isomerase enzyme.

9. A process for engineering a xylose-utilizing bacterial strain selected from the group consisting of *Zymomonas* and *Zymobacter* comprising in any order the steps of:
a) transforming with genes or an operon for expression of transaldolase and transketolase; and
b) transforming with genes or an operon for expression of xylose isomerase and xylulokinase, wherein the xylose isomerase enzyme is expressed from a *Z. mobilis* glyceraldehyde-3-phosphate dehydrogenase gene promoter that has a base substitution in a position selected from the group consisting of position 116, position 217, and both position 116 and 217; wherein the position numbers are of SEQ ID NO: 1; which is an improved P*gap*.

10. A process for production of ethanol, comprising:
culturing in a medium comprising xylose the recombinant bacterial strain of claim 1;
maintaining fermentation conditions suitable for ethanol production in any system,
thereby facilitating the cultured recombinant bacterial strain of claim 1 to convert xylose to ethanol.

11. A recombinant bacterial strain produced according to the process of Claim 8 or 9, wherein the *Zymomonas* or *Zymobacter* is engineered to express xylose isomerase at a level to produce at least about 0.1 μmoles product/mg protein/minute, as determined by reacting 20 μL of cell free extract in a reaction mix, at 30°C, comprising 0.256 mM NADH, 50 mM xylose, 10 mM $MgSO_4$,10 mM thiethanolamine, and 1 U/ml sorbitol dehydrogenase, wherein D-xylulose is the product.

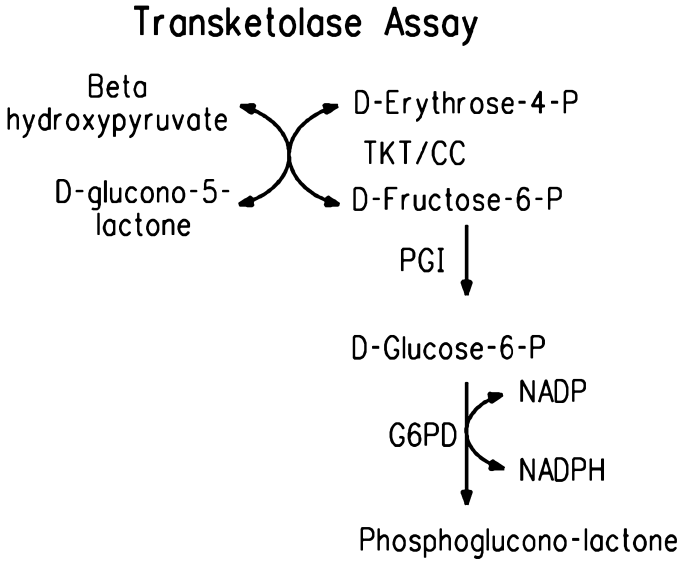

Transketolase Assay
Beta hydroxypyruvate
D-glucono-5-lactone
TKT/CC
D-Erythrose-4-P
D-Fructose-6-P
PGI
D-Glucose-6-P
G6PD
NADP
NADPH
Phosphoglucono-lactone

FIG. 1A

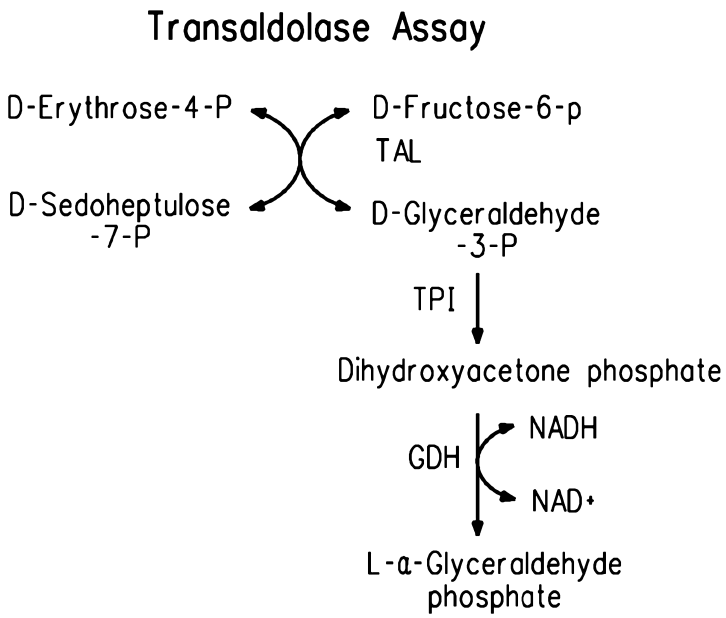

Transaldolase Assay
D-Erythrose-4-P
D-Sedoheptulose -7-P
TAL
D-Fructose-6-p
D-Glyceraldehyde -3-P
TPI
Dihydroxyacetone phosphate
GDH
NADH
NAD+
L-α-Glyceraldehyde phosphate

FIG. 1B

FIG. 1C

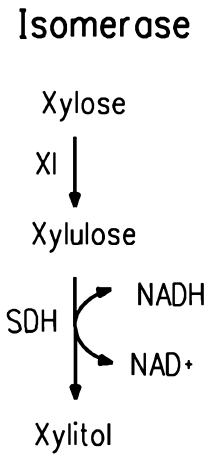
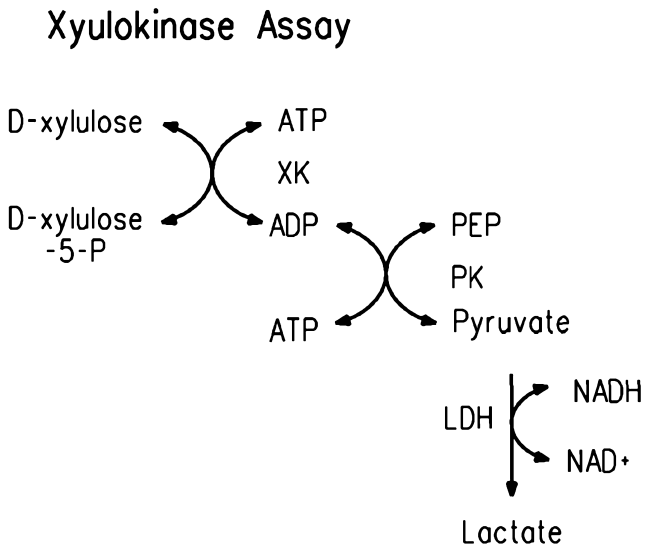

Xylose Isomerase Assay
Xylose
XI
Xylulose
SDH
NADH
NAD+
Xylitol

FIG. 1D


Xyulokinase Assay
D-xylulose
ATP
XK
D-xylulose
-5-P
ADP
PEP
PK
ATP
Pyruvate
LDH
NADH
NAD+
Lactate

XK (μmole/min-mg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
XI (μmole/min-mg)
0.08
0.06
0.04
0.02
0.000
XK
XI
ZW1
8b
ZW1CmX1
ZW1CmX2
T2
T2CCmX1
T2CCmX6
T3
T3CCmX1
T3CCmX2
T3CCmX3
T4
T4CCmX1
T5
T5CCmX1
T5CCmX2

FIG. 2

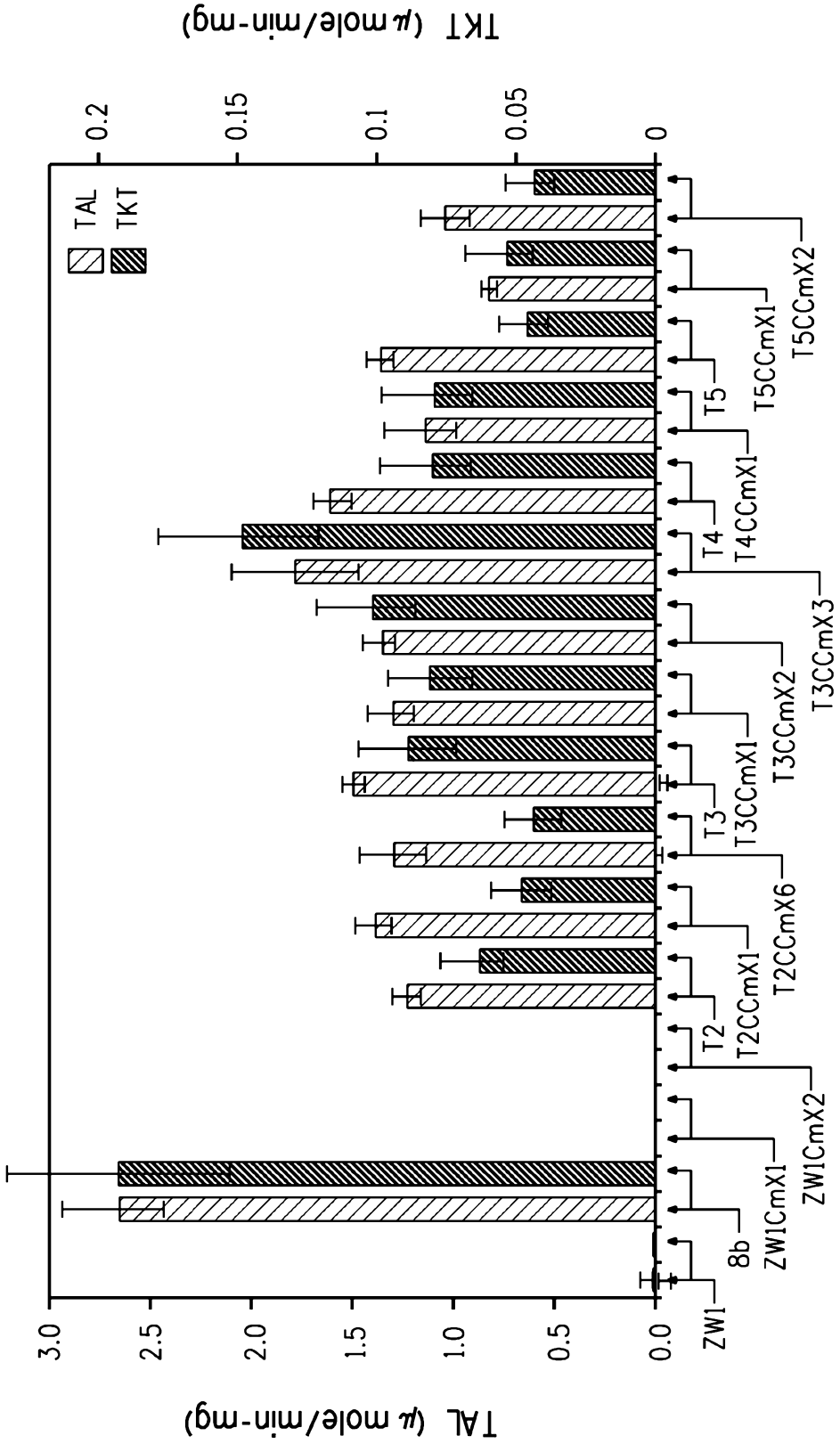

TAL (μ mole/min-mg)
TKT (μmole/min-mg)
TAL
TKT
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
0.05
0.1
0.15
0.2
ZW1
8b
ZW1CmX1
ZW1CmX2
T2
T2CCmX1
T2CCmX6
T3
T3CCmX1
T3CCmX2
T3CCmX3
T4
T4CCmX1
T5
T5CCmX1
T5CCmX2

FIG. 3

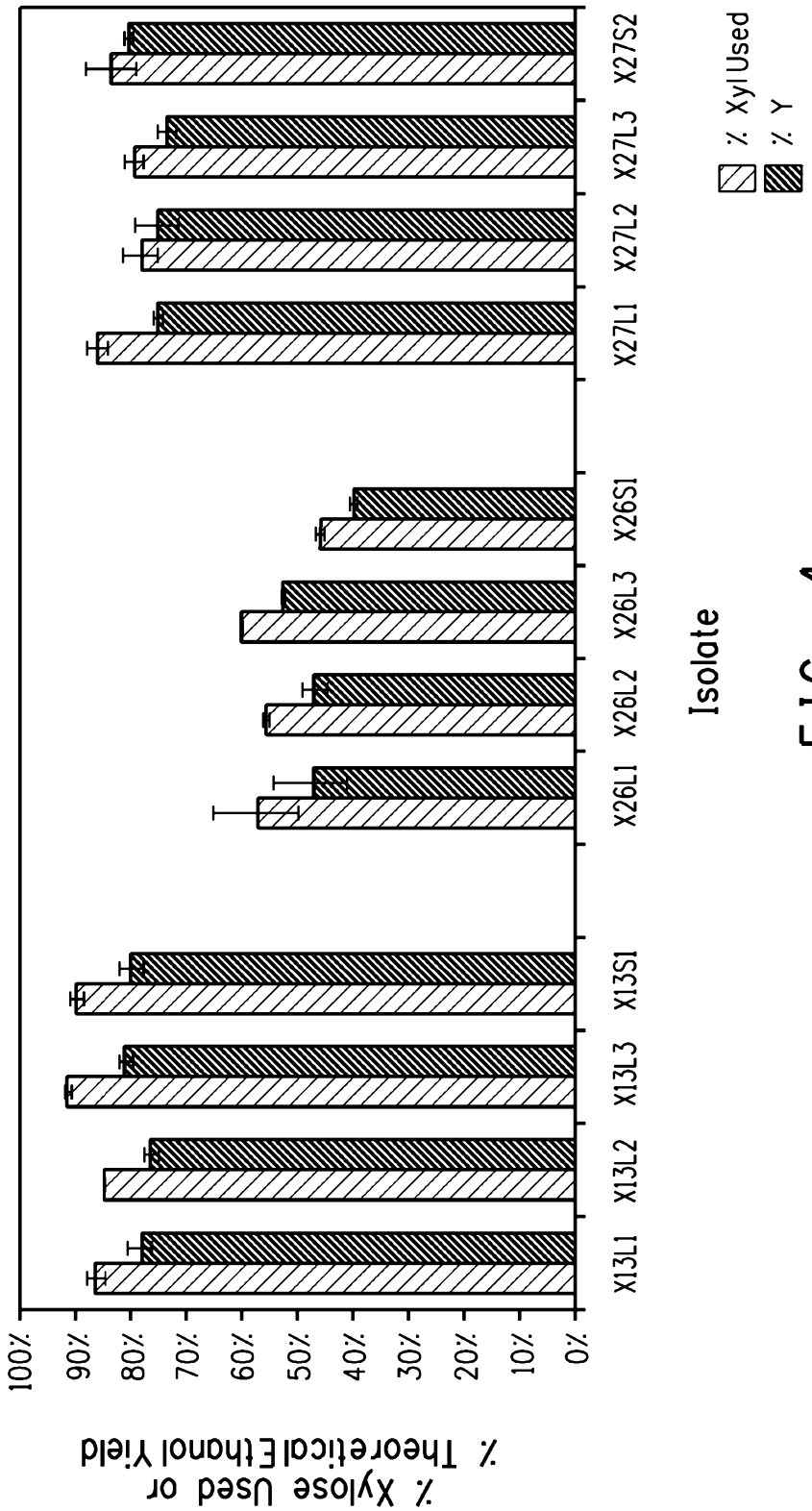

% Xylose Used or
% Theoretical Ethanol Yield
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
X13L1
X13L2
X13L3
X13S1
X26L1
X26L2
X26L3
X26S1
X27L1
X27L2
X27L3
X27S2
Isolate
% Xyl Used
% Y

FIG. 4

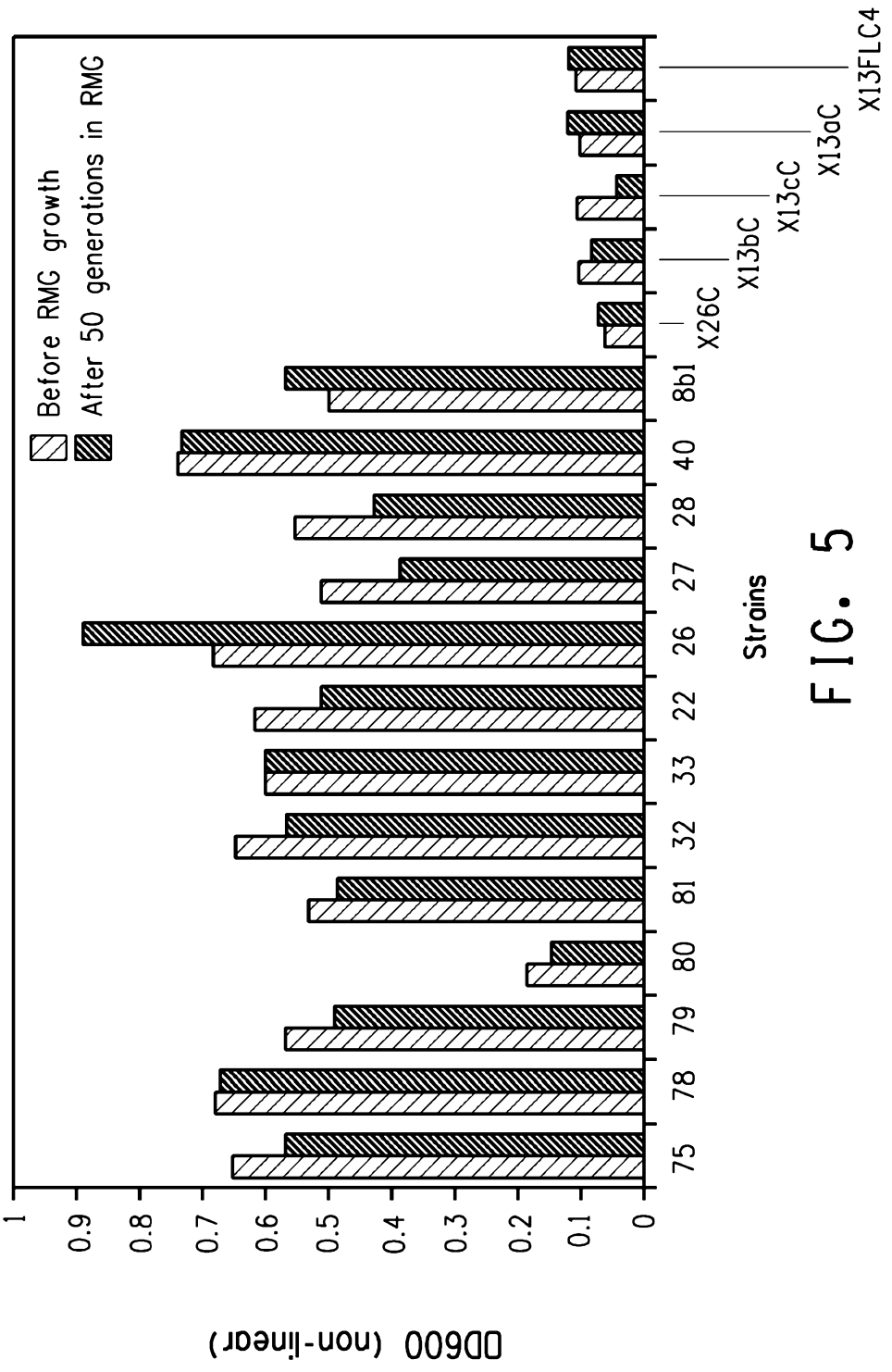

OD600 (non-linear)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Before RMG growth
After 50 generations in RMG
75
78
79
80
81
32
33
22
26
27
28
40
8b1
X26C
X13bC
X13cC
X13aC
X13FLC4
Strains

FIG. 5

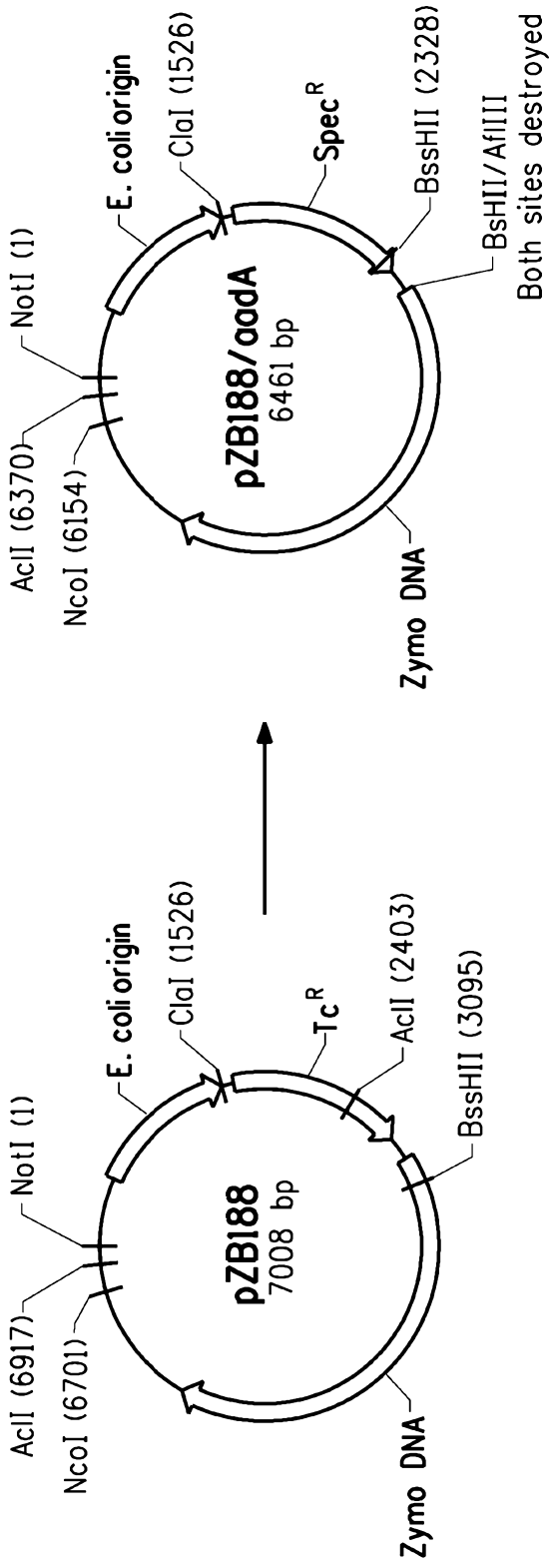



FIG. 6A




FIG. 6B

Continue to Fig. 6C

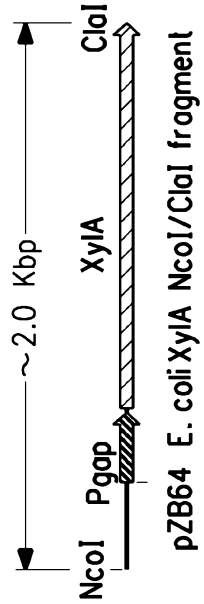

~2.0 Kbp
ClaI
XylA
Pgap
NcoI
pZB64 E. coli XylA NcoI/ClaI fragment

FIG. 6D

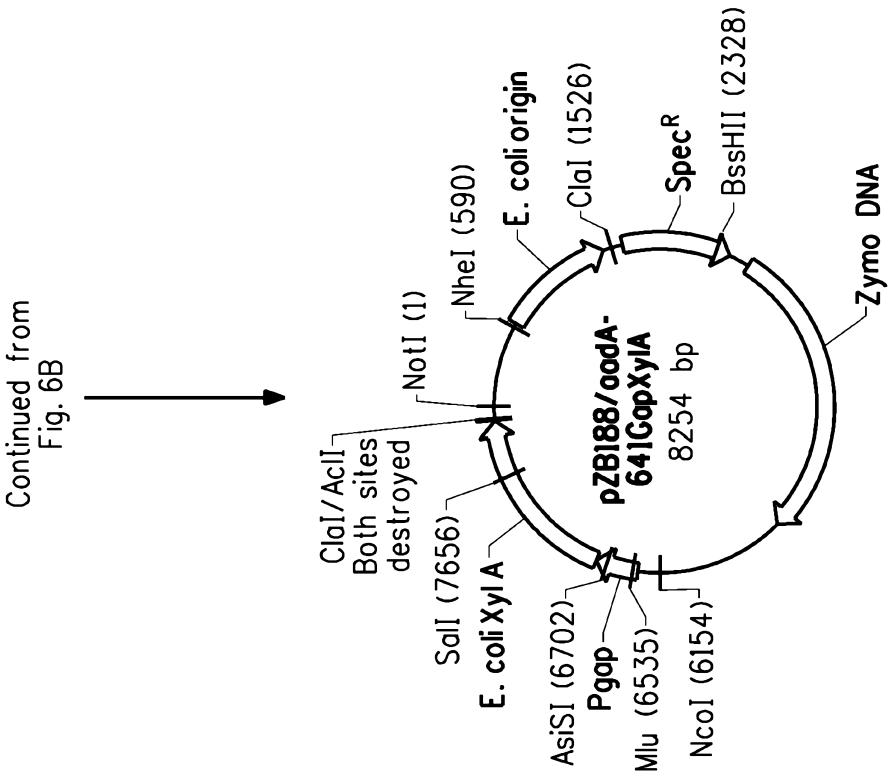

Continued from Fig. 6B
ClaI/AclI Both sites destroyed
NotI (1)
NheI (590)
E. coli origin
ClaI (1526)
Spec^R
BssHII (2328)
Zymo DNA
SalI (7656)
E. coli XylA
AsiSI (6702)
Pgap
MluI (6535)
NcoI (6154)
pZB188/aodA-641GapXylA
8254 bp

FIG. 6C

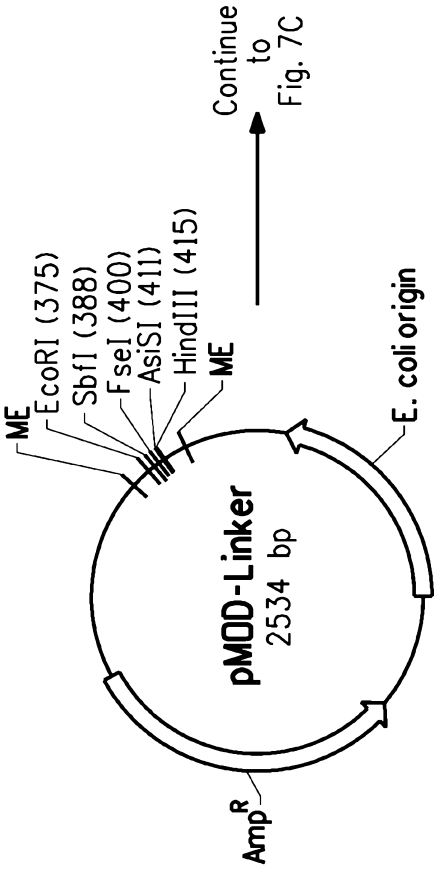

Continue to Fig. 7C
ME
EcoRI (375)
SbfI (388)
FseI (400)
AsiSI (411)
HindIII (415)
ME
E. coli origin
pMOD-Linker
2534 bp
Amp^R

FIG. 7B

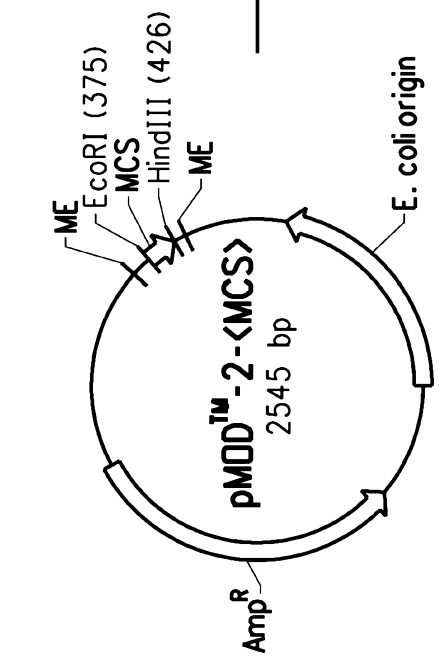

ME
EcoRI (375)
MCS
HindIII (426)
ME
E. coli origin
pMOD™-2-<MCS>
2545 bp
Amp^R

FIG. 7A

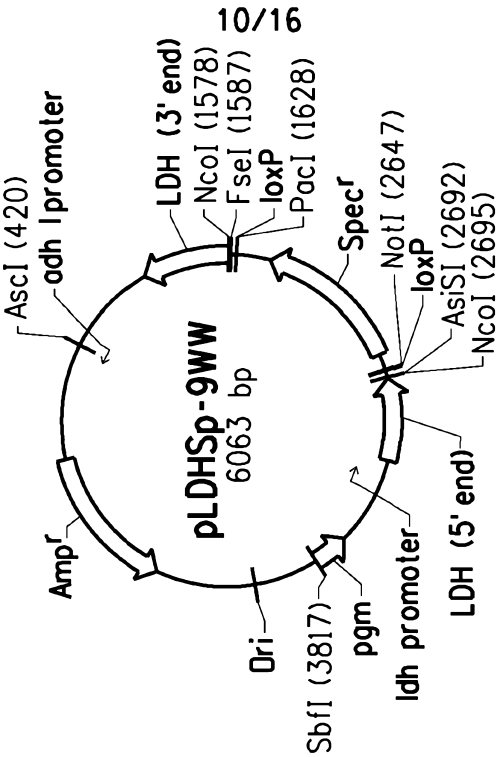

pLDHSp-9WW
6063 bp
AscI (420)
adh I promoter
LDH (3' end)
NcoI (1578)
FseI (1587)
loxP
PacI (1628)
Spec^r
NotI (2647)
loxP
AsiSI (2692)
NcoI (2695)
LDH (5' end)
Idh promoter
pgm
SbfI (3817)
Ori
Amp^r

FIG. 8

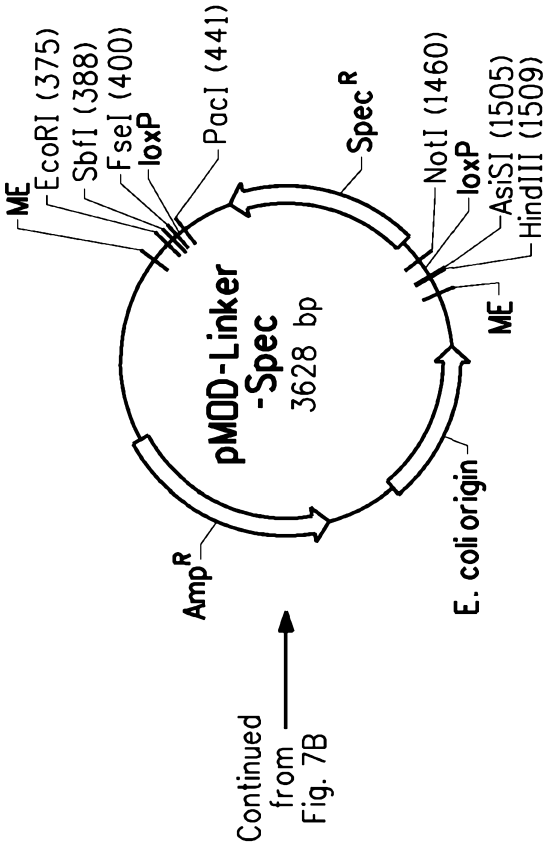

pMOD-Linker
-Spec
3628 bp
ME
EcoRI (375)
SbfI (388)
FseI (400)
loxP
PacI (441)
Spec^R
NotI (1460)
loxP
AsiSI (1505)
HindIII (1509)
ME
E. coli origin
Amp^R
Continued
from
Fig. 7B

FIG. 7C

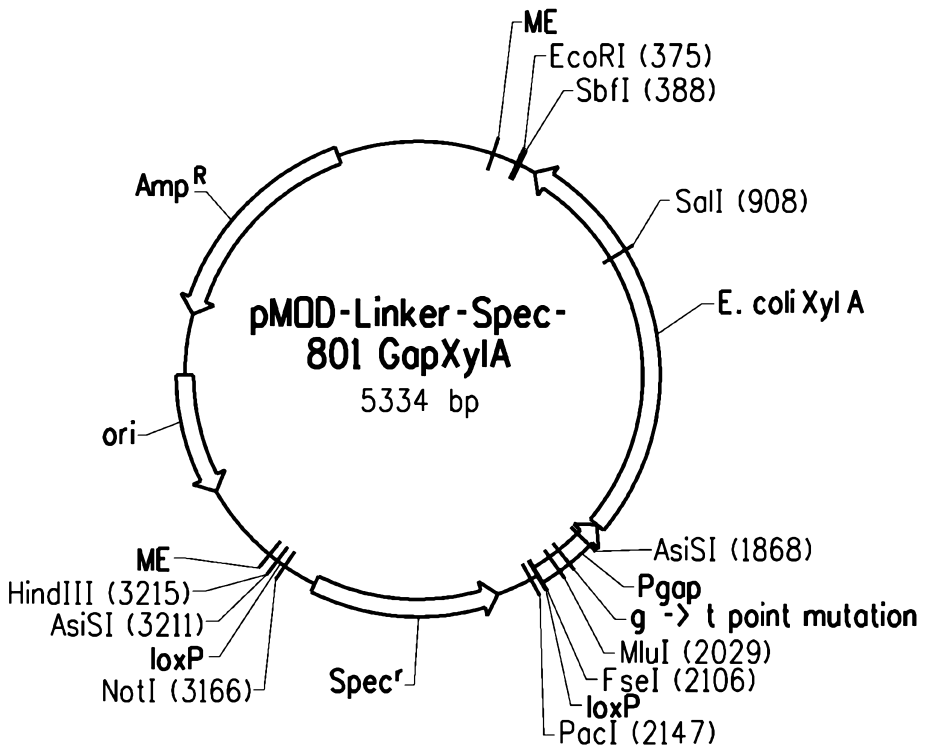

ME
EcoRI (375)
SbfI (388)
SalI (908)
E. coli Xyl A
AsiSI (1868)
Pgap
g -> t point mutation
MluI (2029)
FseI (2106)
loxP
PacI (2147)
Spec^r
ME
HindIII (3215)
AsiSI (3211)
loxP
NotI (3166)
ori
Amp^R
pMOD-Linker-Spec-
801 GapXylA
5334 bp

FIG. 9

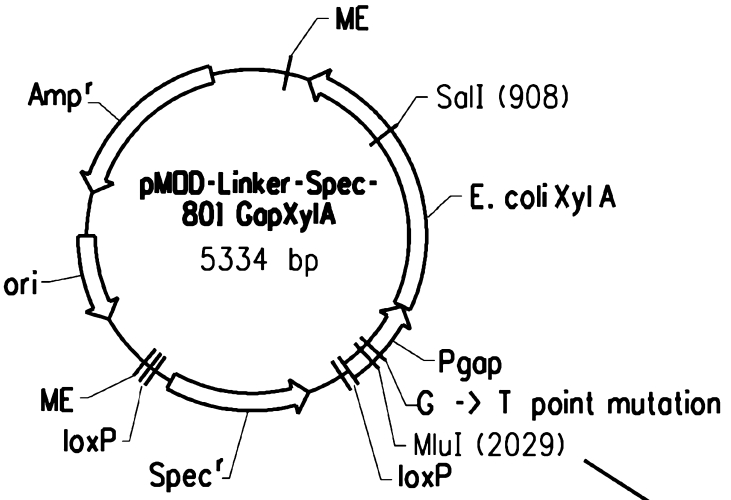



FIG. 10A

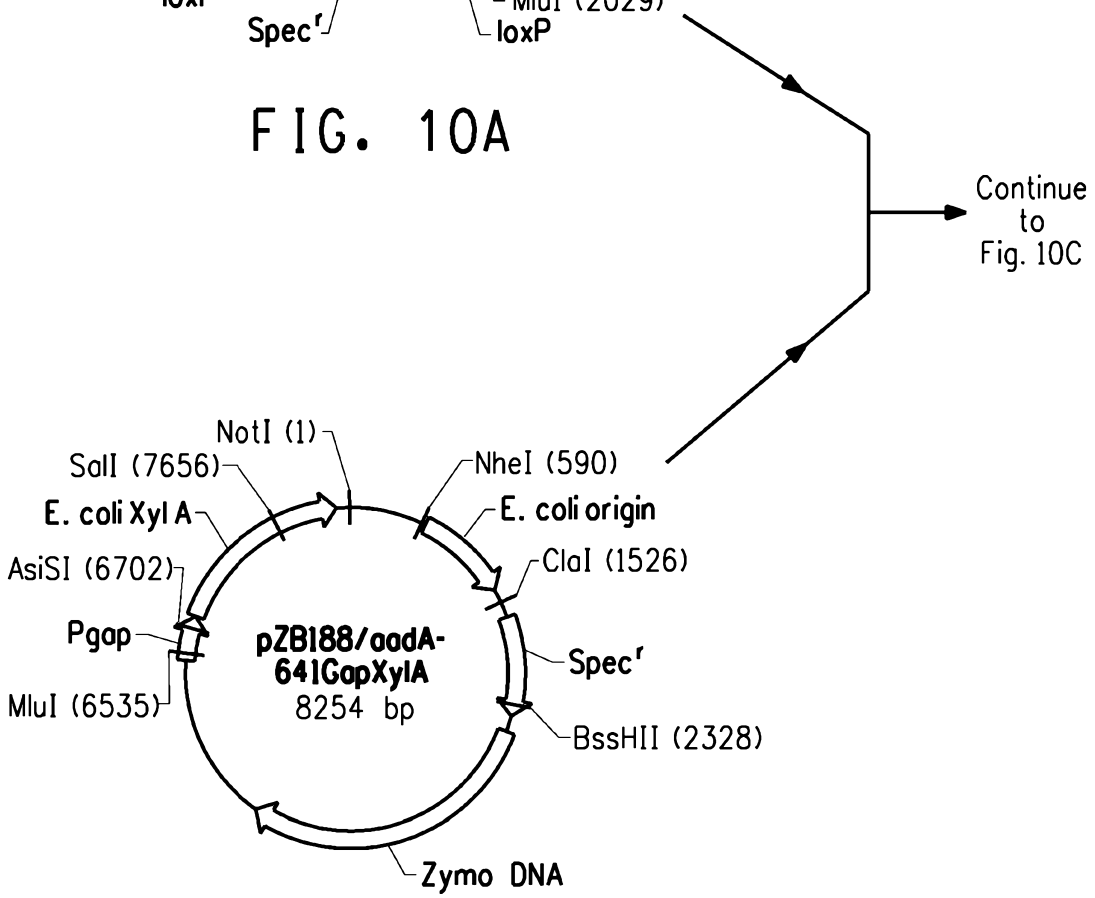



FIG. 10B

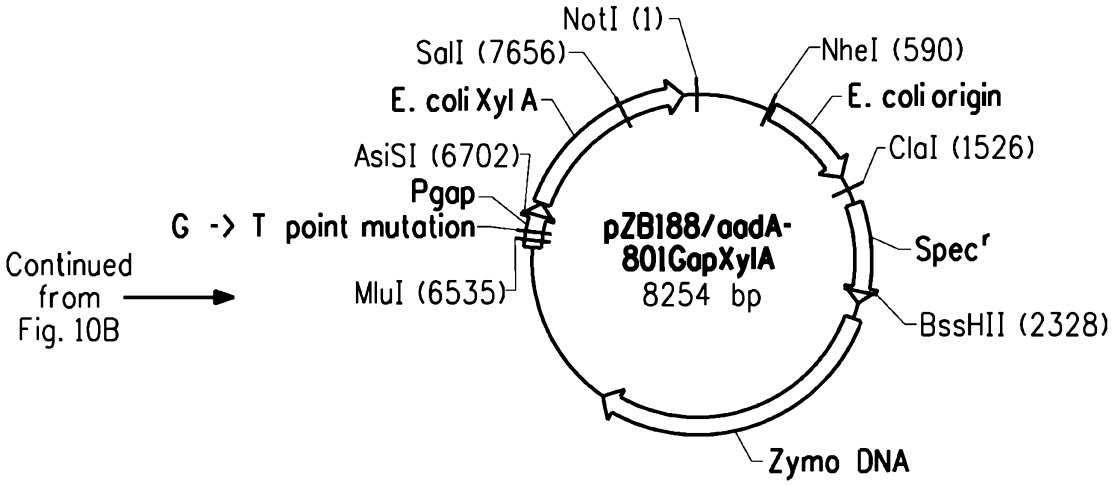

NotI (1)
SalI (7656)
NheI (590)
E. coli Xyl A
E. coli origin
AsiSI (6702)
ClaI (1526)
Pgap
G -> T point mutation
pZB188/odA-801GapXylA
8254 bp
Spec^r
Continued from Fig. 10B
MluI (6535)
BssHII (2328)
Zymo DNA

FIG. 10C

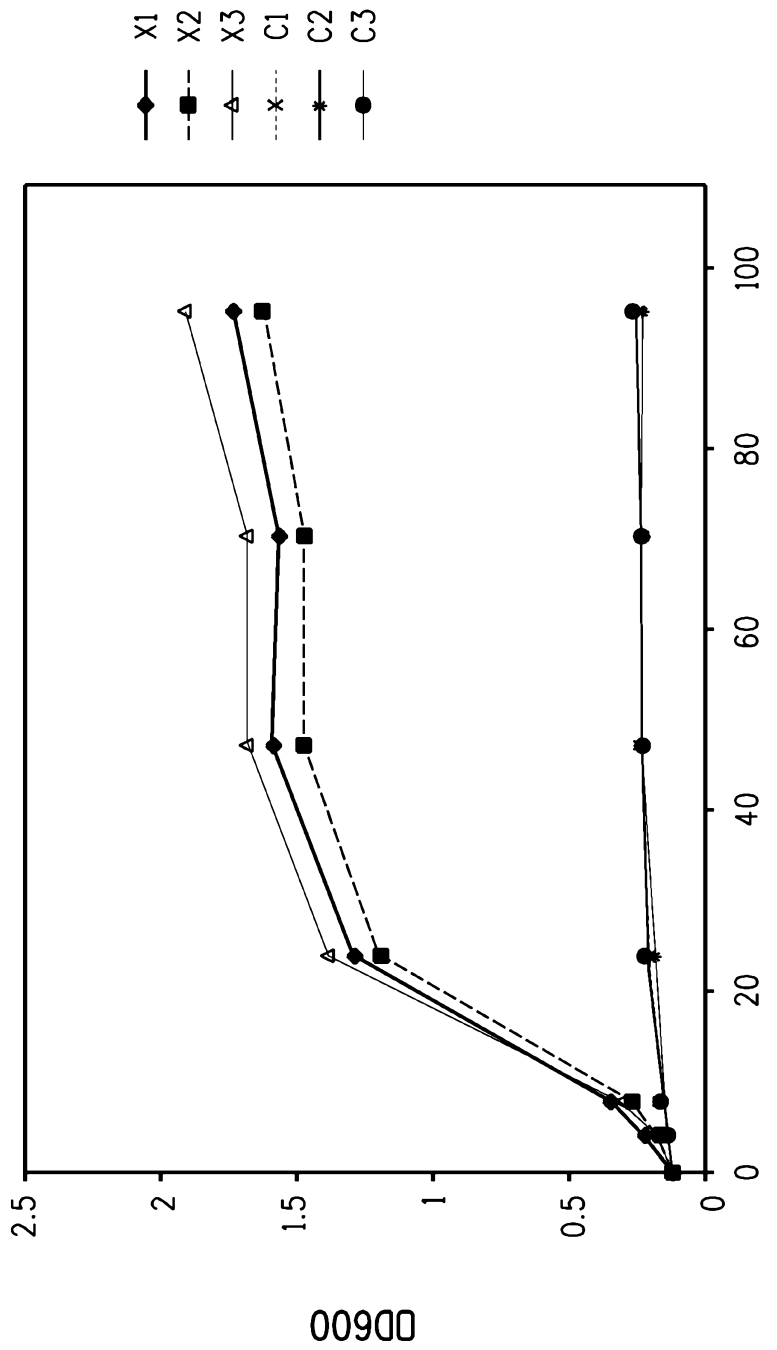



FIG. 11

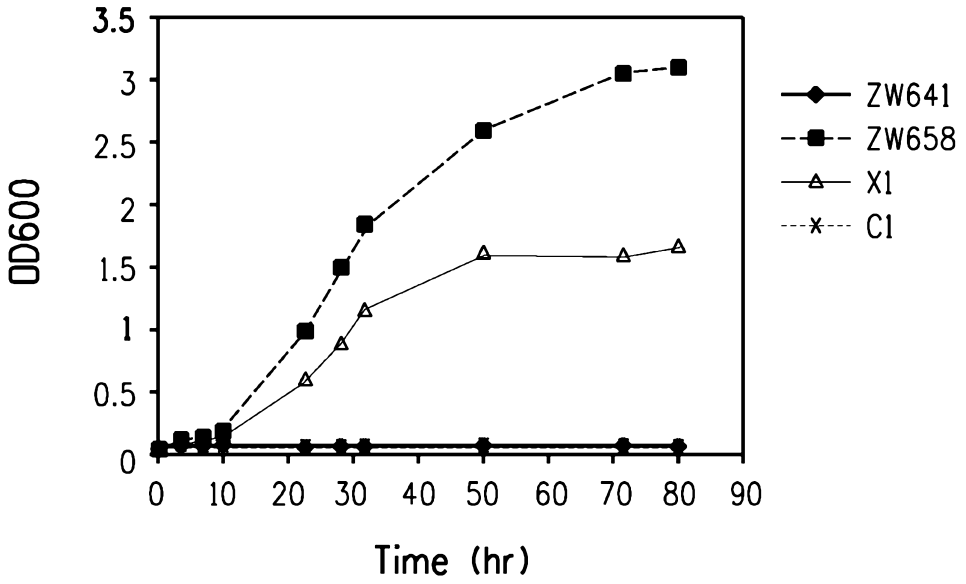



FIG. 12A

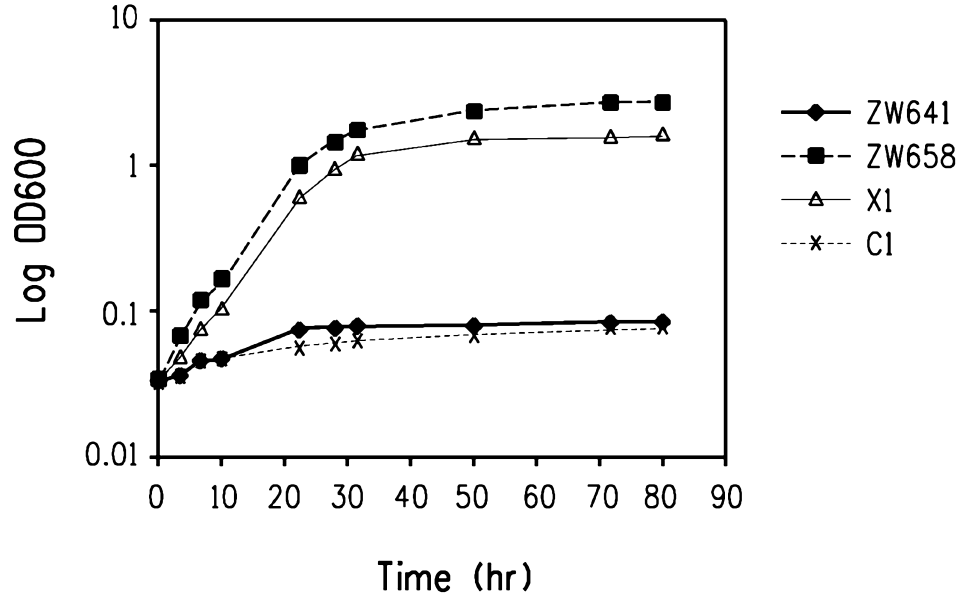



FIG. 12B

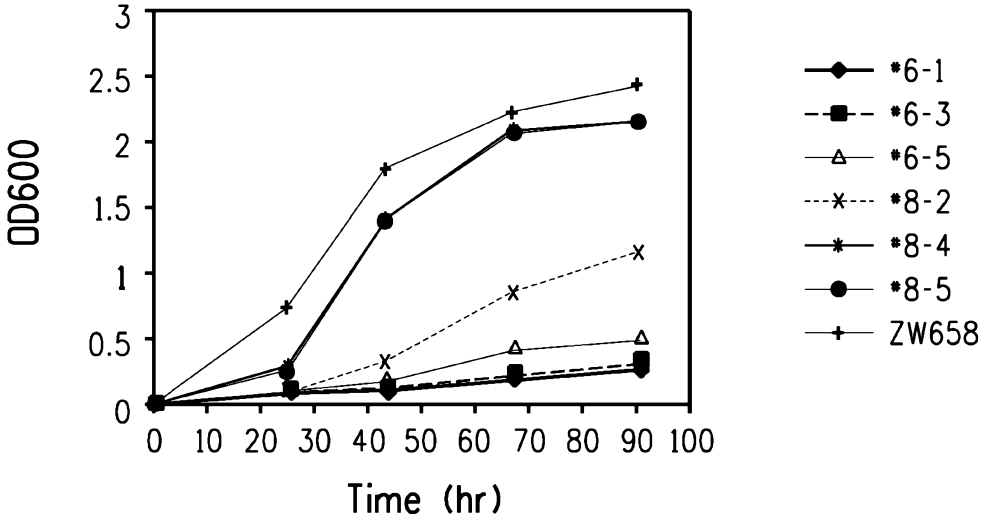



FIG. 13A

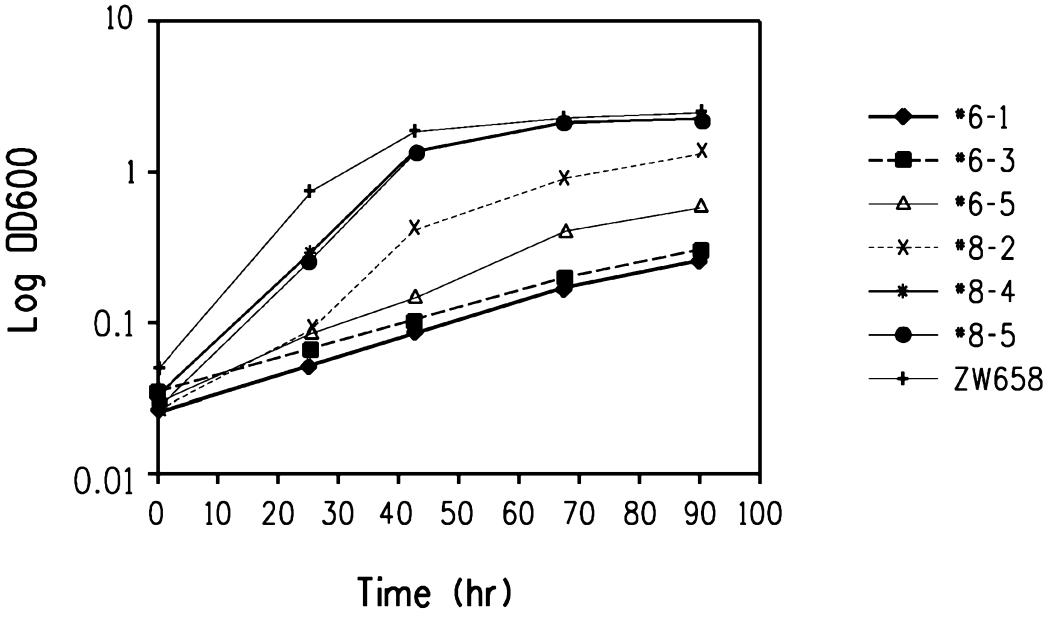



FIG. 13B