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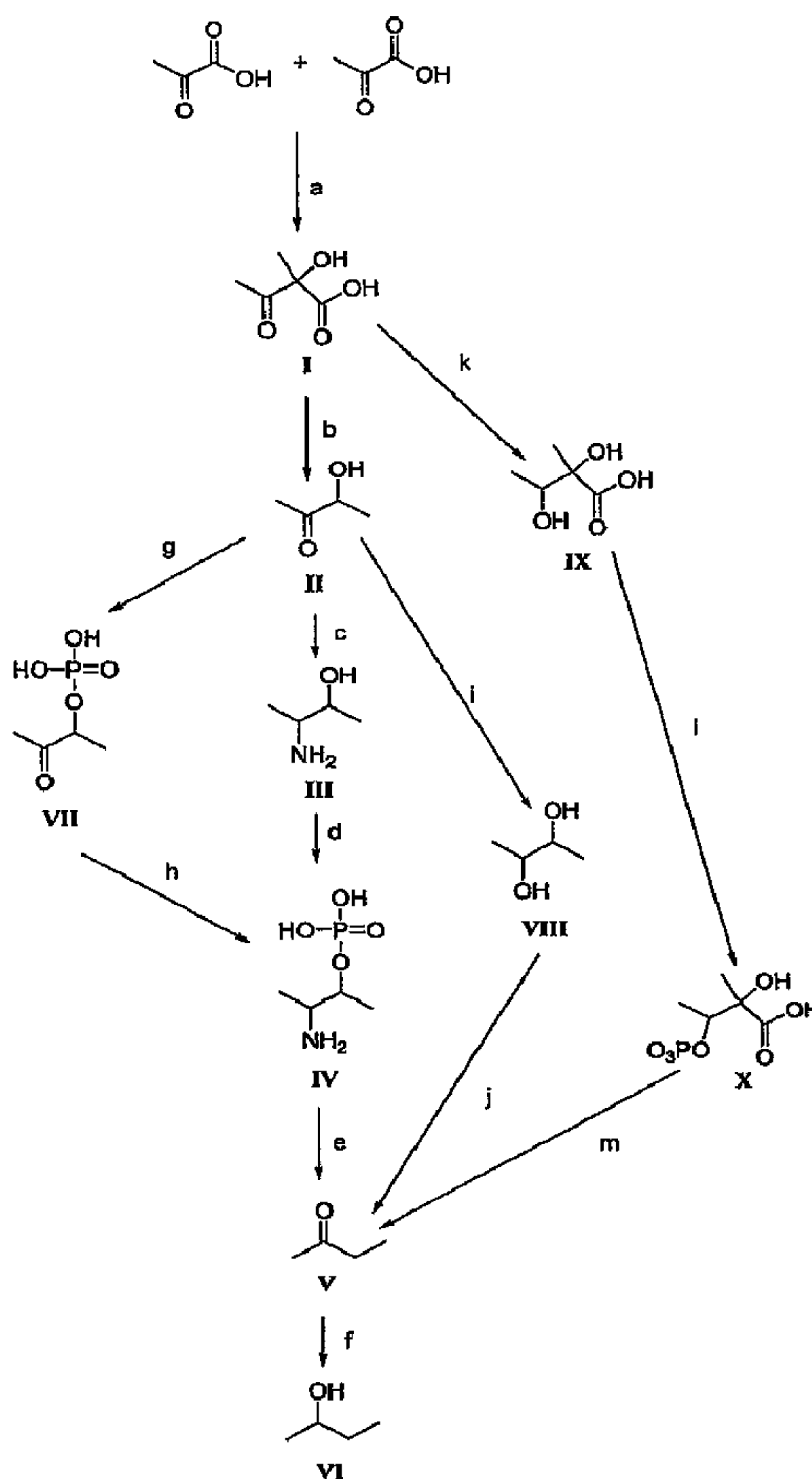
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(54) Title: FERMENTATIVE PRODUCTION OF FOUR CARBON ALCOHOLS



(57) Abrégé/Abstract:

Methods for the fermentative production of four carbon alcohols are provided. Specifically, butanol, preferably 2-butanol is produced by the fermentative growth of a recombinant bacteria expressing a 2- butanol biosynthetic pathway. The recombinant

(57) **Abrégé(suite)/Abstract(continued):**

microorganisms and methods of the invention can also be adapted to produce 2-butanone, an intermediate in the 2-butanol biosynthetic pathways disclosed herein.

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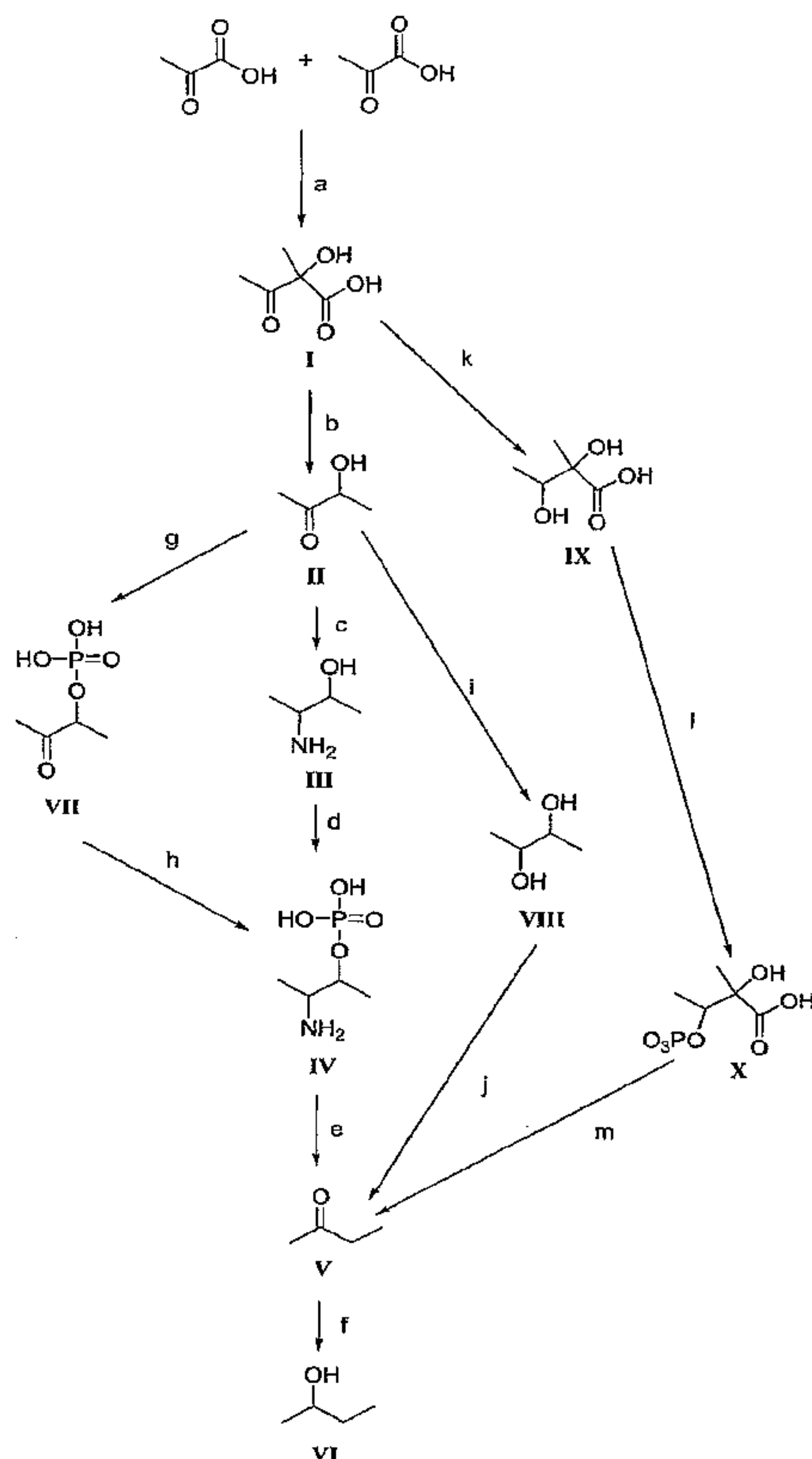
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(54) Title: FERMENTIVE PRODUCTION OF FOUR CARBON ALCOHOLS

(57) Abstract: Methods for the fermentive production of four carbon alcohols are provided. Specifically, butanol, preferably 2-butanol is produced by the fermentive growth of a recombinant bacteria expressing a 2- butanol biosynthetic pathway. The recombinant microorganisms and methods of the invention can also be adapted to produce 2-butanone, an intermediate in the 2-butanol biosynthetic pathways disclosed herein.



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TITLE

FERMENTIVE PRODUCTION OF FOUR CARBON ALCOHOLS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority under 35 U.S.C. §119 from U.S.
5 Provisional Application Serial No. 60/796816, filed May 2, 2006 and U.S.
Provisional Application Serial No. 60/871156, filed December 21, 2006.

FIELD OF THE INVENTION

The invention relates to the field of industrial microbiology and the
production of alcohols. More specifically, 2-butanol is produced via
10 industrial fermentation of a recombinant microorganism. The recombinant
microorganisms and methods of the invention can also be adapted to
produce 2-butanone, an intermediate in the 2-butanol biosynthetic
pathways disclosed herein.

BACKGROUND OF THE INVENTION

15 Butanol is an important industrial chemical, useful as a fuel additive,
as a feedstock chemical in the plastics industry, and as a foodgrade
extractant in the food and flavor industry. Each year 10 to 12 billion
pounds of butanol are produced by petrochemical means and the need for
this commodity chemical will likely increase. 2-Butanone, also referred to
20 as methyl ethyl ketone (MEK), is a widely used solvent and is the most
important commercially produced ketone, after acetone. It is used as a
solvent for paints, resins, and adhesives, as well as a selective extractant
and activator of oxidative reactions.

Methods for the chemical synthesis of 2-butanone are known, such
25 as by dehydrogenation of 2-butanol, or in a process where liquid butane is
catalytically oxidized giving 2-butanone and acetic acid (*Ullmann's
Encyclopedia of Industrial Chemistry*, 6th edition, 2003, Wiley-VCH Verlag
GmbH and Co., Weinheim, Germany, Vol. 5, pp.727-732). 2-Butanone
may also be converted chemically to 2-butanol by hydrogenation (Breen et
30 al., *J. of Catalysis* 236: 270-281 (2005)). Methods for the chemical
synthesis of 2-butanol are known, such as n-butene hydration (*Ullmann's
Encyclopedia of Industrial Chemistry*, 6th edition, 2003, Wiley-VCH Verlag
GmbH and Co., Weinheim, Germany, Vol. 5, pp. 716-719). These

processes use starting materials derived from petrochemicals and are generally expensive, and are not environmentally friendly. The production of 2-butanone and 2-butanol from plant-derived raw materials would minimize greenhouse gas emissions and would represent an advance in the art.

Methods for producing 2-butanol by biotransformation of other organic chemicals are also known. For example, Stampfer et al. (WO 03/078615) describe the production of secondary alcohols, such as 2-butanol, by the reduction of ketones which is catalyzed by an alcohol dehydrogenase enzyme obtained from *Rhodococcus ruber*. Similarly, Kojima et al. (EP 0645453) describe a method for preparing secondary alcohols, such as 2-butanol, by reduction of ketones which is catalyzed by a secondary alcohol dehydrogenase enzyme obtained from *Candida parapsilosis*. Additionally, Kuehnle et al. (EP 1149918) describe a process that produces both 1-butanol and 2-butanol by the oxidation of hydrocarbons by various strains of *Rhodococcus ruber*. The process favored 1-butanol production with a selectivity of 93.8%.

The production of 2-butanol by certain strains of *Lactobacilli* is also known (Speranza et. al. *J. Agric. Food Chem.* (1997) 45:3476-3480). The 2-butanol is produced by the transformation of *meso*-2,3-butanediol. The production of 2-butanol from acetolactate and acetoin by these *Lactobacilli* strains was also demonstrated. However, there have been no reports of a recombinant microorganism designed to produce 2-butanol.

There is a need, therefore, for environmentally responsible, cost-effective processes for the production of 2-butanol and 2-butanone. The present invention addresses this need through the discovery of recombinant microbial production hosts expressing 2-butanol and 2-butanone biosynthetic pathways.

SUMMARY OF THE INVENTION

The invention provides a recombinant microorganism having an engineered 2-butanol biosynthetic pathway. Also provided is a recombinant microorganism having an engineered 2-butanone biosynthetic pathway, which is the same as the 2-butanol biosynthetic

pathway with omission of the last step. The engineered microorganisms may be used for the commercial production of 2-butanol or 2-butanone. Accordingly, the invention provides a recombinant microbial host cell comprising at least one DNA molecule encoding a polypeptide that catalyzes a substrate to product conversion selected from the group consisting of:

- i) pyruvate to alpha-acetolactate;
- ii) alpha-acetolactate to acetoin;
- iii) acetoin to 2,3-butanediol;
- 10 iv) 2,3-butanediol to 2-butanone; and
- v) 2-butanone to 2-butanol;

wherein the at least one DNA molecule is heterologous to said microbial host cell and wherein said microbial host cell produces 2-butanol.

In another embodiment the invention provides a recombinant microbial host cell comprising at least one DNA molecule encoding a polypeptide that catalyzes a substrate to product conversion selected from the group consisting of:

- i) pyruvate to alpha-acetolactate;
- ii) alpha-acetolactate to acetoin;
- 20 iii) acetoin to 2,3-butanediol; and
- iv) 2,3-butanediol to 2-butanone;

wherein the at least one DNA molecule is heterologous to said microbial host cell and wherein said microbial host cell produces 2-butanone.

In another embodiment the invention provides a method for the production of 2-butanol comprising:

- 25 1) providing a recombinant microbial host cell comprising at least one DNA molecule encoding a polypeptide that catalyzes a substrate to product conversion selected from the group consisting of:
 - 30 i) pyruvate to alpha-acetolactate;
 - ii) alpha-acetolactate to acetoin,
 - iii) acetoin to 2,3-butanediol;
 - iv) 2,3-butanediol to 2-butanone; and

v) 2-butanone to 2-butanol;

wherein the at least one DNA molecule is heterologous to said microbial host cell; and

2) contacting the host cell of (1) with a fermentable carbon substrate in a fermentation medium under conditions whereby 2-butanol is produced.

Similarly the invention provides A method for the production of 2-butanone comprising:

1) providing a recombinant microbial host cell comprising at least one DNA molecule encoding a polypeptide that catalyzes a substrate to product conversion selected from the group consisting of:

i) pyruvate to alpha-acetolactate;

ii) alpha-acetolactate to acetoin;

iii) acetoin to 2,3-butanediol; and

iv) 2,3-butanediol to 2-butanone;

wherein the at least one DNA molecule is heterologous to said microbial host cell; and

2) contacting the host cell of (1) with a fermentable carbon substrate in a fermentation medium under conditions whereby 2-butanone is produced.

In another embodiment the invention provides A 2-butanol or 2-butanone containing fermentation product medium produced by the method of the invention.

BRIEF DESCRIPTION OF THE FIGURES , TABLES AND SEQUENCE DESCRIPTIONS

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

Figure 1 shows four different pathways for biosynthesis of 2-butanone and 2-butanol.

Figure 2 shows a phylogenetic tree of full length large subunits of diol/glycerol dehydratases, with >95% identical sequences removed (except that all experimentally verified function sequences were retained),

and a key listing the identity of each sequence in the tree. Sequences with experimentally determined function as diol or glycerol dehydratases are highlighted in dark or light grey, respectively.

Figure 3 shows a phylogenetic tree of full length medium subunits of diol/glycerol dehydratases, with >95% identical sequences removed, and a key listing the identity of each sequence in the tree. Sequences with experimentally determined function as diol or glycerol dehydratases are highlighted in dark or light grey, respectively.

Figure 4 shows a phylogenetic tree of full length small subunits of diol/glycerol dehydratases, with >95% identical sequences removed, and a key listing the identity of each sequence in the tree. Sequences with experimentally determined function as diol or glycerol dehydratases are highlighted in dark or light grey, respectively.

Table 12 is a table of the alpha large subunit Profile HMM for the diol / glycerol dehydratase enzyme. Table 12 is submitted herewith electronically and is incorporated herein by reference.

Table 13 is a table of the beta medium subunit Profile HMM for the diol / glycerol dehydratase enzyme. Table 13 is submitted herewith electronically and is incorporated herein by reference.

Table 14 is a table of the gamma small subunit Profile HMM for the diol / glycerol dehydratase enzyme. Table 14 is submitted herewith electronically and is incorporated herein by reference.

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.

Table 1
Summary of Nucleic Acid and Protein SEQ ID Numbers

Description	SEQ ID Nucleic acid	SEQ ID Protein
<i>budA</i> , acetolactate decarboxylase from <i>Klebsiella pneumoniae</i> ATCC 25955	1	2
<i>alsD</i> , acetolactate decarboxylase from <i>Bacillus subtilis</i>	80	81
<i>budA</i> , acetolactate decarboxylase from <i>Klebsiella terrigena</i>	82	83
<i>budB</i> , acetolactate synthase from <i>Klebsiella pneumoniae</i> ATCC 25955	3	4
<i>alsS</i> , acetolactate synthase from <i>Bacillus subtilis</i>	76	77
<i>budB</i> , acetolactate synthase from <i>Klebsiella terrigena</i>	78	79
<i>budC</i> butanediol dehydrogenase from <i>Klebsiella pneumoniae</i> IAM1063	5	6
butanediol dehydrogenase from <i>Bacillus cereus</i>	84	85
butanediol dehydrogenase from <i>Bacillus cereus</i>	86	87
<i>butB</i> , butanediol dehydrogenase from <i>Lactococcus lactis</i>	88	89
<i>pddA</i> , butanediol dehydratase alpha subunit from <i>Klebsiella oxytoca</i> ATCC 8724	7	8
<i>pddB</i> , butanediol dehydratase beta subunit from <i>Klebsiella oxytoca</i> ATCC 8724	9	10
<i>pddC</i> , butanediol dehydratase gamma subunit from <i>Klebsiella oxytoca</i> ATCC 8724	11	12
<i>pduC</i> , B12 dependent diol dehydratase large subunit from <i>Salmonella typhimurium</i>	92	93
<i>pduD</i> , B12 dependent diol dehydratase medium subunit from <i>Salmonella typhimurium</i>	94	95
<i>pduE</i> , B12 dependent diol dehydratase small subunit from <i>Salmonella typhimurium</i>	96	97
<i>pduC</i> , B12 dependent diol dehydratase large	98	99

subunit from <i>Lactobacillus collinoides</i>		
<i>pduD</i> , B12 dependent diol dehydratase medium subunit from <i>Lactobacillus collinoides</i>	100	101
<i>pduE</i> , B12 dependent diol dehydratase small subunit from <i>Lactobacillus collinoides</i>	102	103
<i>pddC</i> , adenosylcobalamin-dependent diol dehydratase alpha subunit from <i>Klebsiella pneumoniae</i>	104	105
<i>pddD</i> , adenosylcobalamin-dependent diol dehydratase beta subunit from <i>Klebsiella pneumoniae</i>	106	107
<i>pddD</i> , adenosylcobalamin-dependent diol dehydratase gamma subunit from <i>Klebsiella pneumoniae</i>	108	109
<i>ddrA</i> , diol dehydratase reactivating factor large subunit from <i>Klebsiella oxytoca</i>	110	111
<i>ddrB</i> , diol dehydratase reactivating factor small subunit from <i>Klebsiella oxytoca</i>	112	113
<i>pduG</i> , diol dehydratase reactivating factor large subunit from <i>Salmonella typhimurium</i>	114	115
<i>pduH</i> , diol dehydratase reactivating factor small subunit from <i>Salmonella typhimurium</i>	116	117
<i>pduG</i> , diol dehydratase reactivating factor large subunit from <i>Lactobacillus collinoides</i>	118	119
<i>pduH</i> , diol dehydratase reactivating factor small subunit from <i>Lactobacillus collinoides</i>	120	121
<i>sadH</i> , butanol dehydrogenase from <i>Rhodococcus ruber</i> 219	13	14
<i>adhA</i> , butanol dehydrogenase from <i>Pyrococcus furiosus</i>	90	91
<i>chnA</i> , cyclohexanol dehydrogenase from <i>Acintebacter sp.</i>	71	72
<i>yqhD</i> , butanol dehydrogenase from <i>Escherichia coli</i>	74	75
amine:pyruvate transaminase from <i>Vibrio fluvialis</i> (an acetoin aminase)	144 codon	122

	opt.	
amino alcohol kinase from <i>Erwinia carotovora</i> subsp. <i>atroseptica</i>	123	124
amino alcohol O-phosphate lyase from <i>Erwinia carotovora</i> subsp. <i>atroseptica</i>	125	126
<i>budC</i> , acetoin reductase (butanediol dehydrogenase) from <i>Klebsiella terrigena</i> (now <i>Raoultella terrigena</i>)	133	134
glycerol dehydratase alpha subunit from <i>Klebsiella pneumoniae</i>	145	146
glycerol dehydratase beta subunit from <i>Klebsiella pneumoniae</i>	147	148
glycerol dehydratase gamma subunit from <i>Klebsiella pneumoniae</i>	149	150
glycerol dehydratase reactivase large subunit from <i>Klebsiella pneumoniae</i>	151	152
glycerol dehydratase reactivase small subunit from <i>Klebsiella pneumoniae</i>	153	154

SEQ ID NOs:15 - 65 are the nucleotide sequences of oligonucleotide PCR, cloning, screening, and sequencing primers used in the Examples.

5 SEQ ID NO:66 is nucleotide sequence of the deleted region of the *yqhD* gene in *E. coli* strain MG1655 $\Delta yqhCD$, described in Example 11.

SEQ ID NO:67 is the nucleotide sequence of a variant of the glucose isomerase promoter 1.6Gl.

SEQ ID NO:68 is the nucleotide sequence of the 1.5Gl promoter.

10 SEQ ID NO:69 is the nucleotide sequence of the diol dehydratase operon from *Klebsiella oxytoca*.

SEQ ID NO:70 is the nucleotide sequence of the diol dehydratase reactivating factor operon from *Klebsiella oxytoca*.

15 SEQ ID NO:73 is the nucleotide sequence of pDCQ2, which is described in Example 9.

SEQ ID NOs:127 - 132 are the nucleotide sequences of additional oligonucleotide PCR and cloning primers used in the Examples.

SEQ ID NO:155 is a codon optimized coding region for the amino alcohol kinase of *Erwinia carotovora* subsp. *atroseptica*.

5 SEQ ID NO:156 is a codon optimized coding region for the amino alcohol O-phosphate lyase of *Erwinia carotovora* subsp. *atroseptica*.

SEQ ID NOs:157-163 are the nucleotide sequences of additional oligonucleotide PCR and cloning primers used in the Examples.

10 SEQ ID NO:275 is the nucleotide sequence of an operon from *Erwinia carotovora* subsp. *atroseptica*.

Table 2.

Additional glycerol and diol dehydratase large, medium and small subunits

<u>^aDescription</u>	<u>^bsubunit</u>	<u>protein SEQ ID</u>
Corresponding subunits from same organism ^c		
Glycerol dehydratase alpha subunit from <i>Clostridium pasteurianum</i>	L	135
Glycerol dehydratase beta subunit from <i>Clostridium pasteurianum</i>	M	136
Glycerol dehydratase gamma subunit from <i>Clostridium pasteurianum</i>	S	137
Glycerol dehydratase alpha subunit from <i>Escherichia blattae</i>	L	138
Glycerol dehydratase beta subunit from <i>Escherichia blattae</i>	M	139
Glycerol dehydratase gamma subunit from <i>Escherichia blattae</i>	S	140
Glycerol dehydratase alpha subunit from <i>Citrobacter freundii</i>	L	141
Glycerol dehydratase beta subunit from <i>Citrobacter freundii</i>	M	142
Glycerol dehydratase gamma subunit from <i>Citrobacter freundii</i>	S	143
Diol dehydratase alpha subunit from <i>Lactobacillus brevis</i>	L	164

Diol dehydratase beta subunit from <i>Lactobacillus brevis</i>	M	165
Diol dehydratase gamma subunit from <i>Lactobacillus brevis</i>	S	166
Diol dehydratase alpha subunit from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Choleraesuis</i> str. SC-B67	L	167
Diol dehydratase beta subunit from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Choleraesuis</i> str. SC-B67	M	168
Diol dehydratase gamma subunit from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Choleraesuis</i> str. SC-B67	S	169
Propanediol dehydratase, large subunit from <i>Escherichia coli</i> E24377A	L	170
Diol/Glyderol Dehydratase medium subunit from <i>Escherichia coli</i> E24377A	M	171
Propanediol dehydratase, small subunit from <i>Escherichia coli</i> E24377A	S	172
diol dehydratase large subunit from <i>Shigella sonnei</i> Ss046	L	173
diol dehydratase medium subunit from <i>Shigella sonnei</i> Ss046	M	174
diol dehydratase small subunit from <i>Shigella sonnei</i> Ss046	S	175
Propanediol dehydratase large subunit from <i>Yersinia bercovieri</i> ATCC 43970	L	176
hypothetical protein YberA_01000484 from <i>Yersinia bercovieri</i> ATCC 43970	M	177
Propanediol dehydratase small subunit from <i>Yersinia bercovieri</i> ATCC 43970	S	178
Propanediol dehydratase large subunit from <i>Yersinia mollaretii</i> ATCC 43969	L	179
hypothetical protein Ymola_01001292 from <i>Yersinia mollaretii</i> ATCC 43969	M	180
Propanediol dehydratase small subunit from <i>Yersinia mollaretii</i> ATCC 43969	S	181
Diol dehydratase large subunit from <i>Yersinia enterocolitica</i> subsp. <i>enterocolitica</i> 8081	L	182
Diol dehydratase medium subunit from <i>Yersinia enterocolitica</i> subsp. <i>enterocolitica</i> 8081	M	183
diol dehydratase small subunit from <i>Yersinia enterocolitica</i> subsp. <i>enterocolitica</i> 8081	S	184
Propanediol dehydratase large subunit from <i>Yersinia intermedia</i> ATCC 29909	L	185
diol/glycerol dehydratase medium subunit from <i>Yersinia intermedia</i> ATCC 29909	M	186
Propanediol dehydratase small subunit from <i>Yersinia intermedia</i> ATCC 29909	S	187
glycerol dehydratase large subunit from <i>Listeria welshimeri</i> serovar 6b str. SLCC5334	L	188
propanediol utilization dehydratase medium subunit from <i>Listeria welshimeri</i> serovar 6b str. SLCC5334	M	189
propanediol utilization dehydratase small subunit from <i>Listeria welshimeri</i> serovar 6b str. SLCC5334	S	190
hypothetical protein lin1117 from <i>Listeria innocua</i> Clip11262	L	191

hypothetical protein lin1118 from <i>Listeria innocua</i> Clip11262	M	192
hypothetical protein lin1119 from <i>Listeria innocua</i> Clip11262	S	193
hypothetical protein lmo1153 from <i>Listeria monocytogenes</i> EGD-e	L	194
hypothetical protein lmo1154 from <i>Listeria monocytogenes</i> EGD-e	M	195
hypothetical protein lmo1155 from <i>Listeria monocytogenes</i> EGD-e	S	196
glycerol dehydratase large subunit from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhi</i> str. CT18	L	197
diol dehydratase medium subunit from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhi</i> str. CT18	M	198
diol dehydratase small subunit from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhi</i> str. CT18	S	199
putative glycerol dehydratase large subunit [from <i>Escherichia coli</i>	L	200
putative diol dehydratase medium subunit from <i>Escherichia coli</i>	M	201
putative diol dehydratase small subunit from <i>Escherichia coli</i>	S	202
glycerol dehydratase large subunit from <i>Listeria monocytogenes</i> str. 4b F2365	L	203
propanediol utilization: dehydratase, medium subunit from <i>Listeria monocytogenes</i> str. 4b F2365	M	204
propanediol utilization: dehydratase, small subunit from <i>Listeria monocytogenes</i> str. 4b F2365	S	205
Glycerol dehydratase large subunit pduC, putative from <i>Streptococcus sanguinis</i> SK36	L	206
Propanediol utilization: dehydratase medium subunit, putative from <i>Streptococcus sanguinis</i> SK36	M	207
B12-dependent diol dehydratase small subunit, putative from <i>Streptococcus sanguinis</i> SK36	S	208
DhaB from <i>Escherichia blattae</i>	L	209
DhaC from <i>Escherichia blattae</i>	M	210
DhaE from <i>Escherichia blattae</i>	S	211
coenzyme B12-dependent glycerol dehydrogenase large subunit from <i>Clostridium perfringens</i> str. 13	L	212
coenzyme B12-dependent glycerol dehydrogenase medium subunit from <i>Clostridium perfringens</i> str. 13	M	213
coenzyme B12-dependent glycerol dehydrogenase small subunit from <i>Clostridium perfringens</i> str. 13	S	214
Propanediol dehydratase large subunit from <i>Yersinia frederiksenii</i> ATCC 33641	L	215
hypothetical protein YfreA_01000478 from <i>Yersinia</i>	M	216

<i>frederiksenii</i> ATCC 33641]		
Propanediol dehydratase, small subunit from <i>Yersinia frederiksenii</i> ATCC 33641	S	217
Glycerol dehydratase from <i>Thermoanaerobacter ethanolicus</i> X514	L	218
dehydratase medium subunit from <i>Thermoanaerobacter ethanolicus</i> X514	M	219
dehydratase small subunit from <i>Thermoanaerobacter ethanolicus</i> X514	S	220
glycerol dehydratase large subunit GldC from <i>Lactobacillus hilgardii</i>	L	221
glycerol dehydratase medium subunit GldD from <i>Lactobacillus hilgardii</i>	M	222
glycerol dehydratase small subunit GldE from <i>Lactobacillus hilgardii</i>	S	223
Glycerol dehydratase from <i>Lactobacillus reuteri</i> JCM 1112	L	224
similar to diol dehydratase gamma subunit from <i>Lactobacillus reuteri</i> JCM 1112	M	225
Propanediol utilization: dehydratase small subunit from <i>Lactobacillus reuteri</i> JCM 1112	S	226
glycerol dehydratase large subunit GldC from <i>Lactobacillus diolivorans</i>	L	227
glycerol dehydratase medium subunit GldD from <i>Lactobacillus diolivorans</i>	M	228
glycerol dehydratase small subunit GldE from <i>Lactobacillus diolivorans</i>	S	229
propanediol dehydratase large subunit from <i>Lactobacillus reuteri</i>	L	230
propanediol dehydratase medium subunit from <i>Lactobacillus reuteri</i>	M	231
propanediol dehydratase small subunit from <i>Lactobacillus reuteri</i>	S	232
glycerol dehydratase large subunit from <i>Mesorhizobium loti</i> MAFF303099	L+M	233
glycerol dehydratase small subunit from <i>Mesorhizobium</i>	S	234

<i>loti</i> MAFF303099		
Glycerol dehydratase from <i>Mycobacterium vanbaalenii</i> PYR-1	L+M	235
propanediol utilization: dehydratase small subunit from <i>Mycobacterium vanbaalenii</i> PYR-1	S	236
Glycerol dehydratase from <i>Mycobacterium</i> sp. MCS	L+M	237
dehydratase small subunit <i>Mycobacterium</i> sp. MCS	S	238
Dehydratase large subunit: Dehydratase medium subunit from <i>Mycobacterium flavescens</i> PYR-GCK	L+M	239
propanediol utilization: dehydratase, small subunit from <i>Mycobacterium flavescens</i> PYR-GCK	S	240
Glycerol dehydratase from <i>Mycobacterium</i> sp. JLS	L+M	241
dehydratase small subunit from <i>Mycobacterium</i> sp. JLS	S	242
glycerol dehydratase large subunit from <i>Mycobacterium smegmatis</i> str. MC2 155	L	243
dehydratase medium subunit from <i>Mycobacterium smegmatis</i> str. MC2 155	M	244
diol dehydrase gamma subunit from <i>Mycobacterium smegmatis</i> str. MC2 155	S	245
Additional subunits		
glycerol dehydratase large subunit from <i>Mycobacterium smegmatis</i> str. MC2 155	L+M	246
glycerol dehydratase large subunit from <i>Mycobacterium smegmatis</i> str. MC2 155	L+M	247
coenzyme B12-dependent glycerol dehydrogenase small subunit from <i>Mycobacterium smegmatis</i> str. MC2 155	S	248
coenzyme B12-dependent glycerol dehydrogenase small subunit from <i>Mycobacterium smegmatis</i> str. MC2 155	S	249
diol dehydratase medium subunit from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Paratyphi</i> A str. ATCC 9150	M	250
diol dehydratase small subunit from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Paratyphi</i> A str. ATCC 9150	S	251
glycerol dehydratase, beta subunit from <i>Clostridium perfringens</i> SM101	M	252

glycerol dehydrase, gamma subunit from <i>Clostridium perfringens</i> SM101	S	253
PduC from <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhimurium</i>	L	254
glycerol dehydratase large subunit from <i>Listeria monocytogenes</i> str. 4b H7858	L	255
DhaB from <i>Escherichia blattae</i>	L	256
DhaB from uncultured bacterium	L	257
DhaB from uncultured bacterium	L	258
glycerol dehydratase large subunit GldC from <i>Lactobacillus collinoides</i>	L	259
PduD from uncultured bacterium	M	260
PduD from uncultured bacterium	M	261
DhaC from uncultured bacterium	M	262
DhaC from uncultured bacterium	M	263
DhaC from uncultured bacterium	M	264
coenzyme B12-dependent glycerol dehydratase, medium subunit from <i>Clostridium perfringens</i> ATCC 13124	M	265
unknown	M	266
glycerol dehydratase beta subunit from <i>Escherichia blattae</i>	M	267
PduE from uncultured bacterium	S	268
PduE from uncultured bacterium	S	269
dehydratase, small subunit from <i>Listeria monocytogenes</i> str. 1/2a F6854	S	270
DhaE from uncultured bacterium	S	271
DhaE from uncultured bacterium	S	272
DhaE from uncultured bacterium	S	273
dehydratase small subunit from <i>Listeria monocytogenes</i> FSL N1-017	S	274

^aDescription: from the Genbank annotation of the sequence and may not be correct including the glycerol or diol designation, or may not include subunit information.

^bSubunit: identified by sequence homology to the large, medium, or small subunit of the *Klebsiella oxytoca* enzyme.

5

^c Subunits are listed together that are from the same organism and have annotations as the same enzyme, or have Genbank numbers close together indicating proximity in the genome.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for the production of 2-butanol using recombinant microorganisms. The present invention meets a number of commercial and industrial needs. Butanol is an
5 important industrial commodity chemical with a variety of applications, where its potential as a fuel or fuel additive is particularly significant. Although only a four-carbon alcohol, butanol has an energy content similar to that of gasoline and can be blended with any fossil fuel. Butanol is favored as a fuel or fuel additive as it yields only CO₂ and little or no SO_x
10 or NO_x when burned in the standard internal combustion engine. Additionally butanol is less corrosive than ethanol, the most preferred fuel additive to date.

In addition to its utility as a biofuel or fuel additive, butanol has the potential of impacting hydrogen distribution problems in the emerging fuel
15 cell industry. Fuel cells today are plagued by safety concerns associated with hydrogen transport and distribution. Butanol can be easily reformed for its hydrogen content and can be distributed through existing gas stations in the purity required for either fuel cells or combustion engines in vehicles.

20 Finally the present invention produces 2-butanol from plant derived carbon sources, avoiding the negative environmental impact associated with standard petrochemical processes for butanol production.

The present invention also provides recombinant microorganisms and methods for producing 2-butanone, an intermediate in the 2-butanol
25 biosynthetic pathways disclosed herein. 2-Butanone, also known as methyl ethyl ketone (MEK), is useful as a solvent in paints and other coatings. It is also used in the synthetic rubber industry and in the production of paraffin wax.

The following definitions and abbreviations are to be used for the
30 interpretation of the claims and the specification.

The term "invention" or "present invention" as used herein is a non-limiting term and is not intended to refer to any single embodiment of the

particular invention but encompasses all possible embodiments as described in the specification and the claims.

The term "2-butanol biosynthetic pathway" refers to the enzyme pathways to produce 2-butanol from pyruvate.

5 The term "2-butanone biosynthetic pathway" refers to the enzyme pathways to produce 2-butanone from pyruvate.

10 The term "acetolactate synthase", also known as "acetohydroxy acid synthase", refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of two molecules of pyruvic acid to one molecule of alpha-acetolactate. Acetolactate synthase, known as EC 2.2.1.6 [formerly 4.1.3.18] (*Enzyme Nomenclature 1992*, Academic Press, San Diego) may be dependent on the cofactor thiamin pyrophosphate for its activity. Suitable acetolactate synthase enzymes are available from a number of sources, for example, *Bacillus subtilis* [GenBank Nos: AAA22222 NCBI (National Center for Biotechnology Information) amino acid sequence (SEQ ID NO:77), L04470 NCBI nucleotide sequence (SEQ ID NO:76)], *Klebsiella terrigena* [GenBank Nos: AAA25055 (SEQ ID NO:79), L04507 (SEQ ID NO:78)], and *Klebsiella pneumoniae* [GenBank Nos: AAA25079 (SEQ ID NO:4), M73842 (SEQ ID NO:3)].

20 The term "acetolactate decarboxylase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of alpha-acetolactate to acetoin. Acetolactate decarboxylases are known as EC 4.1.1.5 and are available, for example, from *Bacillus subtilis* [GenBank Nos: AAA22223 (SEQ ID NO:81), L04470 (SEQ ID NO:80)], *Klebsiella terrigena* [GenBank Nos: AAA25054 (SEQ ID NO:83), L04507 (SEQ ID NO:82)] and *Klebsiella pneumoniae* [GenBank Nos: AAU43774 (SEQ ID NO:2), AY722056 (SEQ ID NO:1)].

30 The term "acetoin aminase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of acetoin to 3-amino-2-butanol. Acetoin aminase may utilize the cofactor pyridoxal 5'-phosphate or NADH (reduced nicotinamide adenine dinucleotide) or NADPH (reduced nicotinamide adenine dinucleotide phosphate). The resulting product may have (*R*) or (*S*) stereochemistry at

the 3-position. The pyridoxal phosphate-dependent enzyme may use an amino acid such as alanine or glutamate as the amino donor. The NADH- and NADPH-dependent enzymes may use ammonia as a second substrate. A suitable example of an NADH-dependent acetoin aminase, also known as amino alcohol dehydrogenase, is described by Ito et al. (U.S. Patent No. 6,432,688). An example of a pyridoxal-dependent acetoin aminase is the amine:pyruvate aminotransferase (also called amine:pyruvate transaminase) described by Shin and Kim (*J. Org. Chem.* 67:2848-2853 (2002)).

10 The term "butanol dehydrogenase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the interconversion of 2-butanone and 2-butanol. Butanol dehydrogenases are a subset of a broad family of alcohol dehydrogenases. Butanol dehydrogenase may be NAD- or NADP-dependent. The NAD-dependent enzymes are known as
15 EC 1.1.1.1 and are available, for example, from *Rhodococcus ruber* [GenBank Nos: CAD36475 (SEQ ID NO:14), AJ491307 (SEQ ID NO:13)]. The NADP-dependent enzymes are known as EC 1.1.1.2 and are available, for example, from *Pyrococcus furiosus* [GenBank Nos: AAC25556 (SEQ ID NO:91), AF013169 (SEQ ID NO:90)]. Additionally, a
20 butanol dehydrogenase is available from *Escherichia coli* [GenBank Nos:NP_417484 (SEQ ID NO:75), NC_000913 (SEQ ID NO:74)] and a cyclohexanol dehydrogenase is available from *Acinetobacter sp.* [GenBank Nos: AAG10026 (SEQ ID NO:72), AF282240 (SEQ ID NO:71)].

 The term "acetoin kinase" refers to a polypeptide (or polypeptides)
25 having an enzyme activity that catalyzes the conversion of acetoin to phosphoacetoin. Acetoin kinase may utilize ATP (adenosine triphosphate) or phosphoenolpyruvate as the phosphate donor in the reaction. Although there are no reports of enzymes catalyzing this reaction on acetoin, there are enzymes that catalyze the analogous reaction on the similar substrate
30 dihydroxyacetone, for example, enzymes known as EC 2.7.1.29 (Garcia-Alles et al. (2004) *Biochemistry* 43:13037-13046).

 The term "acetoin phosphate aminase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of

phosphoacetoin to 3-amino-2-butanol O-phosphate. Acetoin phosphate aminase may use the cofactor pyridoxal 5'-phosphate, NADH or NADPH. The resulting product may have (*R*) or (*S*) stereochemistry at the 3-position. The pyridoxal phosphate-dependent enzyme may use an amino acid such as alanine or glutamate. The NADH- and NADPH-dependent enzymes may use ammonia as a second substrate. Although there are no reports of enzymes catalyzing this reaction on phosphoacetoin, there is a pyridoxal phosphate-dependent enzyme that is proposed to carry out the analogous reaction on the similar substrate serinol phosphate (Yasuta et al. (2001) *Appl. Environ. Microbiol.* 67:4999-5009).

The term "aminobutanol phosphate phospho-lyase", also called "amino alcohol O-phosphate lyase", refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of 3-amino-2-butanol O-phosphate to 2-butanone. Aminobutanol phosphate phospho-lyase may utilize the cofactor pyridoxal 5'-phosphate. There are no previous reports of enzymes catalyzing this reaction on aminobutanol phosphate, though there are reports of enzymes that catalyze the analogous reaction on the similar substrate 1-amino-2-propanol phosphate (Jones et al. (1973) *Biochem J.* 134:167-182). The present invention describes a newly identified aminobutanol phosphate phospho-lyase (SEQ ID NO: 126) from the organism *Erwinia carotovora*, with the activity demonstrated in Example 15 herein.

The term "aminobutanol kinase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of 3-amino-2-butanol to 3-amino-2-butanol O-phosphate. Aminobutanol kinase may utilize ATP as the phosphate donor. Although there are no reports of enzymes catalyzing this reaction on 3-amino-2-butanol, there are reports of enzymes that catalyze the analogous reaction on the similar substrates ethanolamine and 1-amino-2-propanol (Jones et al., *supra*). The present invention describes, in Example 14, an amino alcohol kinase of *Erwinia carotovora* subsp. *atroseptica* (SEQ ID NO:124). The term "butanediol dehydrogenase" also known as "acetoin reductase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the

conversion of acetoin to 2,3-butanediol. Butanediol dehydrogenases are a subset of the broad family of alcohol dehydrogenases. Butanediol dehydrogenase enzymes may have specificity for production of (*R*) - or (*S*)-stereochemistry in the alcohol product. (*S*)-specific butanediol dehydrogenases are known as EC 1.1.1.76 and are available, for example, from *Klebsiella pneumoniae* (GenBank Nos: BBA13085 (SEQ ID NO:6), D86412 (SEQ ID NO:5)). (*R*)-specific butanediol dehydrogenases are known as EC 1.1.1.4 and are available, for example, from *Bacillus cereus* [GenBank Nos. NP_830481 (SEQ ID NO:85), NC_004722 (SEQ ID NO:84); AAP07682 (SEQ ID NO:87), AE017000 (SEQ ID NO:86)], and *Lactococcus lactis* [GenBank Nos. AAK04995 (SEQ ID NO:89), AE006323 (SEQ ID NO:88)].

The term "butanediol dehydratase", also known as "diol dehydratase" or "propanediol dehydratase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of 2,3-butanediol to 2-butanone. Butanediol dehydratase may utilize the cofactor adenosyl cobalamin (vitamin B12). Adenosyl cobalamin-dependent enzymes are known as EC 4.2.1.28 and are available, for example, from *Klebsiella oxytoca* [GenBank Nos: BAA08099 (alpha subunit) (SEQ ID NO:8), D45071 (SEQ ID NO:7); BAA08100 (beta subunit) (SEQ ID NO:10), D45071 (SEQ ID NO:9); and BBA08101 (gamma subunit) (SEQ ID NO:12), D45071 (SEQ ID NO:11) (Note all three subunits are required for activity)], and *Klebsiella pneumoniae* [GenBank Nos: AAC98384 (alpha subunit) (SEQ ID NO:105), AF102064 (SEQ ID NO:104); GenBank Nos: AAC98385 (beta subunit) (SEQ ID NO:107), AF102064 (SEQ ID NO:106), GenBank Nos: AAC98386 (gamma subunit) (SEQ ID NO:109), AF102064 (SEQ ID NO:108)]. Other suitable diol dehydratases include, but are not limited to, B12-dependent diol dehydratases available from *Salmonella typhimurium* [GenBank Nos: AAB84102 (large subunit) (SEQ ID NO:93), AF026270 (SEQ ID NO:92); GenBank Nos: AAB84103 (medium subunit) (SEQ ID NO:95), AF026270 (SEQ ID NO:94); GenBank Nos: AAB84104 (small subunit) (SEQ ID NO:97), AF026270 (SEQ ID NO:96)]; and *Lactobacillus collinoides*

[GenBank Nos: CAC82541 (large subunit) (SEQ ID NO:99), AJ297723 (SEQ ID NO:98); GenBank Nos: CAC82542 (medium subunit) (SEQ ID NO:101); AJ297723 (SEQ ID NO:100); GenBank Nos: CAD01091 (small subunit) (SEQ ID NO:103), AJ297723 (SEQ ID NO:102)]; and enzymes
5 from *Lactobacillus brevis* (particularly strains CNRZ 734 and CNRZ 735, Speranza et al., *supra*), and nucleotide sequences that encode the corresponding enzymes. Methods of diol dehydratase gene isolation are well known in the art (e.g., U.S. Patent No. 5,686,276). Additional diol dehydratases are listed in Table 2.

10 The term "glycerol dehydratase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of glycerol to 3-hydroxypropionaldehyde. Adenosyl cobalamin-dependent glycerol dehydratases are known as EC 4.2.1.30. The glycerol dehydratases of EC 4.2.1.30 are similar to the diol dehydratases in
15 sequence and in having three subunits. The glycerol dehydratases can also be used to convert 2,3-butanediol to 2-butanone. Some examples of glycerol dehydratases of EC 4.2.1.30 include those from *Klebsiella pneumoniae* (alpha subunit, SEQ ID NO:145, coding region and SEQ ID NO:146, protein; beta subunit, SEQ ID NO:147, coding region and SEQ ID
20 NO:148, protein; and gamma subunit SEQ ID NO:149, coding region and SEQ ID NO:150, protein); from *Clostridium pasteurianum* [GenBank Nos: 3360389 (alpha subunit, SEQ ID NO:135), 3360390 (beta subunit, SEQ ID NO:136), and 3360391 (gamma subunit, SEQ ID NO:137)]; from *Escherichia blattae* [GenBank Nos: 60099613 (alpha subunit, SEQ ID
25 NO:138), 57340191 (beta subunit, SEQ ID NO:139), and 57340192 (gamma subunit, SEQ ID NO:140)]; and from *Citrobacter freundii* [GenBank Nos: 1169287 (alpha subunit, SEQ ID NO:141), 1229154 (beta subunit, SEQ ID NO:142), and 1229155 (gamma subunit, SEQ ID NO:143)]. Note that all three subunits are required for activity. Additional
30 glycerol dehydratases are listed in Table 2.

Diol and glycerol dehydratases may undergo suicide inactivation during catalysis. A reactivating factor protein, also referred to herein as "reactivase", can be used to reactivate the inactive enzymes (Mori et al., *J.*

Biol. Chem. 272:32034 (1997)). Preferably, the reactivating factor is obtained from the same source as the diol or glycerol dehydratase used. For example, suitable diol dehydratase reactivating factors are available from *Klebsiella oxytoca* [GenBank Nos: AAC15871 (large subunit) (SEQ ID NO:111), AF017781 (SEQ ID NO:110); GenBank Nos: AAC15872 (small subunit) (SEQ ID NO:113), AF017781 (SEQ ID NO:112)]; *Salmonella typhimurium* [GenBank Nos: AAB84105 (large subunit) (SEQ ID NO:115), AF026270 (SEQ ID NO:114), GenBank Nos: AAD39008 (small subunit) (SEQ ID NO:117), AF026270 (SEQ ID NO:116)]; and *Lactobacillus collinoides* [GenBank Nos: CAD01092 (large subunit) (SEQ ID NO:119), AJ297723 (SEQ ID NO:118); GenBank Nos: CAD01093 (small subunit) (SEQ ID NO:121), AJ297723 (SEQ ID NO:120)]. Both the large and small subunits are required for activity. For example, suitable glycerol dehydratase reactivating factors are available from *Klebsiella pneumoniae* (large subunit, SEQ ID NO:151, coding region and SEQ ID NO:152, protein;, and small subunit, SEQ ID NO:153, coding region and SEQ ID NO:154, protein).

The term "a facultative anaerobe" refers to a microorganism that can grow in both aerobic and anaerobic environments.

The term "carbon substrate" or "fermentable carbon substrate" refers to a carbon source capable of being metabolized by host organisms of the present invention and particularly carbon sources selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, and one-carbon substrates or mixtures thereof.

The term "gene" refers to a nucleic acid fragment that is capable of being expressed as a specific protein, optionally including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native 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" or "heterologous" 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. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

As used herein, an "isolated nucleic acid fragment" or "isolated nucleic acid molecule" or "genetic construct" will be used interchangeably and will mean 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 fragment 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 T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) 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 T_m 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
5 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
10 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
15 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
20 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
25 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
30 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.

5 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
10 equivalent to any of the nucleic acid sequences disclosed herein.

 "Codon degeneracy" refers to the nature in the genetic code permitting variation of the nucleotide sequence without effecting 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
15 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 "percent identity", as known in the art, is a relationship
20 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"
25 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
30 Griffin, H. G., Eds.) Humana: 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

5 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

10 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.

15 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

20 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.*

25 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

30 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.

As used herein the term "coding sequence" or "CDS" refers to a DNA sequence that codes for a specific amino acid sequence. "Suitable regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing site, effector binding site and stem-loop structure.

The term "promoter" 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 or physiological conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

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 effecting 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 "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide.

5 As used herein the term "transformation" refers to the transfer of a nucleic acid fragment into a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms.

10 The terms "plasmid" and "vector" 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 fragments. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear or
15 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. "Transformation
20 vector" refers to a specific vector containing a foreign gene and having elements in addition to the foreign gene that facilitates transformation of a particular host cell.

 As used herein the term "codon degeneracy" refers to the nature in the genetic code permitting variation of the nucleotide sequence without
25 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
30 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 "fermentation product medium" refers to a medium in which fermentation has occurred such that product is present in the
5 medium.

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*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
10 (1989) (hereinafter "Maniatis"); and by Silhavy, T. J., Bennis, M. L. and Enquist, L. W., *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory Press, 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 (1987).

15 The 2-Butanol and 2-Butanone Biosynthetic Pathways

Carbohydrate utilizing microorganisms employ the Embden-Meyerhof-Parnas (EMP) pathway, the Entner-Doudoroff pathway and the pentose phosphate cycle as the central, metabolic routes to provide energy and cellular precursors for growth and maintenance. These
20 pathways have in common the intermediate glyceraldehyde-3-phosphate, and, ultimately, pyruvate is formed directly or in combination with the EMP pathway. The combined reactions of sugar conversion to pyruvate produce energy (e.g. adenosine-5'-triphosphate, ATP) and reducing equivalents (e.g. reduced nicotinamide adenine dinucleotide, NADH, and
25 reduced nicotinamide adenine dinucleotide phosphate, NADPH). NADH and NADPH must be recycled to their oxidized forms (NAD^+ and NADP^+ , respectively). In the presence of inorganic electron acceptors (e.g. O_2 , NO_3^- and SO_4^{2-}), the reducing equivalents may be used to augment the energy pool; alternatively, a reduced carbon by-product may be formed.

30 The invention enables the production of 2-butanone or 2-butanol from carbohydrate sources with recombinant microorganisms by providing a complete biosynthetic pathway from pyruvate to 2-butanone or 2-butanol. Three additional pathways are described. Although 2-butanol is

not known to be the major product of any bacterial fermentation, there are a number of possible pathways for the production of 2-butanol via known biochemical reaction types. These pathways are shown in Figure 1. The letters and roman numerals cited below correspond to the letters and
 5 roman numerals in Figure 1, which are used to depict the conversion steps and products, respectively. As described below, 2-butanone is an intermediate in all of these 2-butanol biosynthetic pathways.

All of the pathways begin with the initial reaction of two pyruvate molecules to yield alpha-acetolactate (I), shown as the substrate to
 10 product conversion (a) in Figure 1. From alpha-acetolactate, there are 4 possible pathways to 2-butanone (V), referred to herein as 2-butanone biosynthetic pathways:

- Pathway 1) I--->II--->III--->IV--->V (substrate to product conversions
 15 b,c,d,e);
 2) I--->II--->VII--->IV--->V (substrate to product conversions b,g,h,e)
 3) I--->II--->VIII--->V (substrate to product conversions b,i,j):
 This is the pathway of the present invention.
 20 4) I--->IX--->X--->V (substrate to product conversions k,l,m)

The 2-butanol biosynthetic pathways conclude with the conversion of 2-butanone (V) to 2-butanol (VI). A detailed discussion of the substrate to product conversions in each pathway is given below.

25 Pathway 1:

(a) pyruvate to alpha-acetolactate:

The initial step in pathway 1 is the conversion of two molecules of pyruvate to one molecule of alpha-acetolactate (compound I in Figure 1) and one molecule of carbon dioxide catalyzed by a thiamin
 30 pyrophosphate-dependent enzyme. Enzymes catalyzing this substrate to product conversion (generally called either acetolactate synthase or acetohydroxy acid synthase; EC 2.2.1.6 [switched from 4.1.3.18 in 2002]) are well-known, and they participate in the biosynthetic pathway for the

proteinogenic amino acids leucine and valine, as well as in the pathway for fermentative production of 2,3-butanediol and acetoin of a number of organisms.

The skilled person will appreciate that polypeptides having
5 acetolactate synthase activity isolated from a variety of sources will be useful in the present invention independent of sequence homology. Some example of suitable acetolactate synthase enzymes are available from a number of sources, for example, *Bacillus subtilis* [GenBank Nos: AAA22222 NCBI (National Center for Biotechnology Information) amino
10 acid sequence (SEQ ID NO:77), L04470 NCBI nucleotide sequence (SEQ ID NO:76)], *Klebsiella terrigena* [GenBank Nos: AAA25055 (SEQ ID NO:79), L04507 (SEQ ID NO:78)], and *Klebsiella pneumoniae* [GenBank Nos: AAA25079 (SEQ ID NO:4), M73842 (SEQ ID NO:3)]. Preferred acetolactate synthase enzymes are those that have at least 80% – 85%
15 identity to SEQ ID NO's 4, 77, and 79, where at least 85% - 90% identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix, is most preferred.

20 (b) alpha-acetolactate to acetoin:

Alpha-acetolactate (I) is converted to acetoin (II) by the action of an enzyme such as acetolactate decarboxylase (EC 4.1.1.5). Like acetolactate synthase, this enzyme is thiamin pyrophosphate-dependent and is also involved in the production of 2,3-butanediol and acetoin by a
25 number of organisms. The enzymes from different sources vary quite widely in size (25-50 kilodaltons), oligomerization (dimer-hexamer), localization (intracellular of extracellular), and allosteric regulation (for example, activation by branched-chain amino acids). For the purpose of the present invention, an intracellular location is preferable to extracellular,
30 but other variations are generally acceptable.

The skilled person will appreciate that polypeptides having acetolactate decarboxylase activity isolated from a variety of sources will be useful in the present invention independent of sequence homology.

Some example of suitable acetolactate decarboxylase enzymes are available from a number of sources, for example, *Bacillus subtilis* [GenBank Nos: AAA22223 (SEQ ID NO:81), L04470 (SEQ ID NO:80)], *Klebsiella terrigena* [GenBank Nos: AAA25054 (SEQ ID NO:83), L04507 (SEQ ID NO:82)] and *Klebsiella pneumoniae* [GenBank Nos: AAU43774 (SEQ ID NO:2), AY722056 (SEQ ID NO:1)].

Preferred acetolactate decarboxylase enzymes are those that have at least 80% – 85% identity to SEQ ID NO's 2, 81 and 83, where at least 85% - 90% identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix, is most preferred.

(c) acetoin to 3-amino-2-butanol:

There are two known types of biochemical reactions that could effect the substrate to product conversion of acetoin (II) to 3-amino-2-butanol (III), specifically, pyridoxal phosphate-dependent transamination utilizing an accessory amino donor and direct reductive amination with ammonia. In the latter case, the reducing equivalents are supplied in the form of a reduced nicotinamide cofactor (either NADH or NADPH). An example of an NADH-dependent enzyme catalyzing this reaction with acetoin as a substrate is reported by Ito et al. (U.S. Patent No. 6,432,688). Any stereospecificity of this enzyme has not been assessed. An example of a pyridoxal phosphate-dependent transaminase that catalyzes the conversion of acetoin to 3-amino-2-butanol has been reported by Shin and Kim (*supra*). This enzyme was shown in Example 13 herein to convert both the (*R*) isomer of acetoin to the (2*R*,3*S*) isomer of 3-amino-2-butanol and the (*S*) isomer of acetoin to the (2*S*,3*S*) isomer of 3-amino-2-butanol. Either type of enzyme (i.e., transaminase or reductive aminase) is considered to be an acetoin aminase and may be utilized in the production of 2-butanol. Other enzymes in this group may have different stereospecificities.

The skilled person will appreciate that polypeptides having acetoin aminase activity isolated from a variety of sources will be useful in the

present invention independent of sequence homology. One example of this activity has is described herein and is identified as SEQ ID NO:122. Accordingly preferred acetoin aminase enzymes are those that have at least 80% – 85% identity to SEQ ID NO:122, where at least 85% - 90% identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix, is most preferred.

(d) 3-amino-2-butanol to 3-amino-2-butanol O-phosphate:

There are no enzymes known in the art that catalyze the substrate to product conversion of 3-amino-2-butanol (III) to 3-amino-2-butanol phosphate (IV). However, a few *Pseudomonas* and *Erwinia* species have been shown to express an ATP-dependent ethanolamine kinase (EC 2.7.1.82) which allows them to utilize ethanolamine or 1-amino-2-propanol as a nitrogen source (Jones et al. (1973) *Biochem. J.* 134:167-182). It is likely that this enzyme also has activity towards 3-amino-2-butanol or could be engineered to do so, thereby providing an aminobutanol kinase. The present invention describes in Example 14 a gene of *Erwinia carotovora* subsp. *atroseptica* (SEQ ID NO:123) that encodes a protein (SEQ ID NO:24) that is identified as an amino alcohol kinase. This enzyme may be used to convert 3-amino-2-butanol to 3-amino-2-butanol O-phosphate.

The skilled person will appreciate that polypeptides having aminobutanol kinase activity isolated from a variety of sources will be useful in the present invention independent of sequence homology. One example of this activity has is described herein and is identified as SEQ ID NO:124. Accordingly preferred aminobutanol kinase enzymes are those that have at least 80% – 85% identity to SEQ ID NO:124, where at least 85% - 90% identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix, is most preferred.

(e) 3-amino-2-butanol phosphate to 2-butanone:

Although there are no enzymes reported to catalyze the substrate to product conversion of 3-amino-2-butanol phosphate (IV) to 2-butanone (V), the substrate is very similar to those utilized by the pyridoxal phosphate-dependent phosphoethanolamine phospho-lyase enzyme, which has been found in a small number of *Pseudomonas* and *Erwinia* species. These enzymes have activity towards phosphoethanolamine and both enantiomers of 2-phospho-1-aminopropane (Jones et al. (1973) *Biochem. J.* 134:167-182), and may also have activity towards 3-amino-2-butanol O-phosphate. The present invention describes a gene of *Erwinia carotovora* subsp. *atroseptica* (SEQ ID NO:125) that encodes a protein (SEQ ID NO:126) with homology to class III aminotransferases. Example 15 demonstrates that this enzyme is active on both aminopropanol phosphate and aminobutanol phosphate substrates. The newly identified and characterized enzyme was able to catalyze the conversion of a mixture of (*R*)-3-amino-(*S*)-2-butanol and (*S*)-3-amino-(*R*)-2-butanol O-phosphate, and a mixture of (*R*)-3-amino-(*R*)-2-butanol and (*S*)-3-amino-(*S*)-2-butanol O-phosphate to 2-butanone. The newly identified and characterized enzyme was also able to catalyze the conversion of both (*R*) and (*S*)-2-amino-1-propanol phosphate to propanone, with a preference for (*S*)-2-amino-1-propanol phosphate. The highest activity was observed with the proposed natural substrate DL-1-amino-2-propanol phosphate, which was converted to propionaldehyde.

The skilled person will appreciate that polypeptides having aminobutanol phosphate phospho-lyase activity isolated from a variety of sources will be useful in the present invention independent of sequence homology. One example of a suitable aminobutanol phosphate phospho-lyase enzyme is described herein as SEQ ID NO: 126. Accordingly preferred aminobutanol phosphate phospho-lyase enzymes are those that have at least 80% – 85% identity to SEQ ID NO's 126, where at least 85% - 90% identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default parameters

of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix, is most preferred.

(f) 2-butanone to 2-butanol:

The final step in all pathways to produce 2- butanol from pyruvic
5 acid is the reduction of 2-butanone (V) to 2-butanol (VI). This substrate to product conversion is catalyzed by some members of the broad class of alcohol dehydrogenases (types utilizing either NADH or NADPH as a source of hydride, depending on the enzyme) that may be called butanol dehydrogenases. Enzymes of each type that catalyze the reduction of 2-
10 butanone are well known, as described above in the definition for butanol dehydrogenase.

The skilled person will appreciate that polypeptides having butanol dehydrogenase activity isolated from a variety of sources will be useful in the present invention independent of sequence homology. Some example
15 of suitable butanol dehydrogenase enzymes are available from a number of sources, for example, *Rhodococcus ruber* [GenBank Nos: CAD36475 (SEQ ID NO:14), AJ491307 (SEQ ID NO:13)]. The NADP-dependent enzymes are known as EC 1.1.1.2 and are available, for example, from *Pyrococcus furiosus* [GenBank Nos: AAC25556 (SEQ ID NO:91),
20 AF013169 (SEQ ID NO:90)]. Additionally, a butanol dehydrogenase is available from *Escherichia coli* [GenBank Nos:NP_417484 (SEQ ID NO:75), NC_000913 (SEQ ID NO:74)] and a cyclohexanol dehydrogenase is available from *Acinetobacter* sp. [GenBank Nos: AAG10026 (SEQ ID NO:72), AF282240 (SEQ ID NO:71)]. Preferred butanol dehydrogenase
25 enzymes are those that have at least 80% – 85% identity to SEQ ID NO's 14, 91, 75, and 72, where at least 85% - 90% identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix,
30 is most preferred.

Pathway 2:(a) pyruvate to alpha-acetolactate:

This substrate to product conversion is the same as described above for Pathway 1.

5 (b) alpha-acetolactate to acetoin:

This substrate to product conversion is the same as described above for Pathway 1.

(g) acetoin to phosphoacetoin:

Although enzymes that catalyze the substrate to product conversion
10 of acetoin (II) to phosphoacetoin (VII) have not been described, the structure of the substrate acetoin is very similar to that of dihydroxyacetone, and therefore acetoin may be an acceptable substrate for dihydroxyacetone kinase (EC 2.7.1.29), an enzyme which catalyzes phosphorylation of dihydroxyacetone. Protein engineering techniques for
15 the alteration of substrate specificity of enzymes are well known (Antikainen and Martin (2005) *Bioorg. Med. Chem.* 13:2701-2716) and may be used to generate an enzyme with the required specificity. In this conversion, the phosphate moiety may be supplied by any high energy biological phosphate donor, with the common substrates being
20 phosphoenolpyruvate (as in the *E. coli* dihydroxyacetone kinase) and ATP (as in the *Citrobacter freundii* dihydroxyacetone kinase) (Garcia-Alles et al. (2004) *Biochemistry* 43:13037-13045).

(h) phosphoacetoin to 3-amino-2-butanol O-phosphate:

Although enzymes that catalyze the substrate to product conversion
25 of phosphoacetoin (VII) to 3-amino-2-butanol O-phosphate (IV) have not been described, the structure of the substrate is very similar to that of dihydroxyacetone phosphate, a substrate for the proposed serinol phosphate aminotransferase encoded by the 5' portion of the *rtxA* gene in some species of *Bradyrhizobium* (Yasuta et al., *supra*). Thus a serinol
30 phosphate aminotransferase may be functional in this step.

(e) 3-amino-2-butanol O-phosphate to 2-butanone:

This substrate to product conversion is the same as described above for Pathway 1.

(f) 2-butanone to 2-butanol:

This substrate to product conversion is the same as described above for Pathway 1.

Pathway 3:

5 (a) pyruvate to alpha-acetolactate:

This substrate to product conversion is the same as described above for Pathway 1.

(b) alpha-acetolactate to acetoin:

10 This substrate to product conversion is the same as described above for Pathway 1.

(i) acetoin to 2,3-butanediol:

The substrate to product conversion of acetoin (II) to 2,3-butanediol (VIII) may be catalyzed by a butanediol dehydrogenase that may either utilize NADH or NADPH as the source of reducing equivalents when
15 carrying out reductions. Enzymes with activity towards acetoin participate in the pathway for production of 2,3-butanediol in organisms that produce that compound. The reported enzymes (e.g., BudC from *Klebsiella pneumoniae* (Ui et al. (2004) *Letters in Applied Microbiology* 39:533-537) generally utilize NADH. Either cofactor is acceptable for use in the
20 production of 2-butanol by this pathway.

The skilled person will appreciate that polypeptides having butanediol dehydrogenase activity isolated from a variety of sources will be useful in the present invention independent of sequence homology. Some example of suitable butanediol dehydrogenase enzymes are
25 available from a number of sources, for example, *Klebsiella pneumoniae* (GenBank Nos: BBA13085 (SEQ ID NO:6), D86412 (SEQ ID NO:5)). (*R*)-specific butanediol dehydrogenases are known as EC 1.1.1.4 and are available, for example, from *Bacillus cereus* [GenBank Nos. NP_830481 (SEQ ID NO:85), NC_004722 (SEQ ID NO:84); AAP07682 (SEQ ID
30 NO:87), AE017000 (SEQ ID NO:86)], and *Lactococcus lactis* [GenBank Nos. AAK04995 (SEQ ID NO:89), AE006323 (SEQ ID NO:88)]. Preferred butanediol dehydrogenases enzymes are those that have at least 80% – 85% identity to SEQ ID NO's 6, 85, 87, and 89, where at least 85% - 90%

identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix, is most preferred.

5 (j) 2,3-butanediol to 2-butanone:

The substrate to product conversion of 2,3-butanediol (VIII) to 2-butanone (V) may be catalyzed by diol dehydratase enzymes (EC 4.2.1.28) and glycerol dehydratase enzymes (EC 4.2.1.30). The best characterized diol dehydratase is the coenzyme B12-dependent *Klebsiella*
10 *oxytoca* enzyme, but similar enzymes are found in a number of enteric bacteria. The *K. oxytoca* enzyme has been shown to accept *meso*-2,3-butanediol as a substrate (Bachovchin *et al.* (1977) *Biochemistry* 16:1082-1092), producing the desired product 2-butanone. Example 17 demonstrates that the *Klebsiella pneumoniae* glycerol dehydratase was
15 able to convert *meso*-2,3-butanediol to 2-butanone. The three subunit of the *Klebsiella pneumoniae* glycerol dehydratase (alpha: SEQ ID NO:145 (coding region) and 146 (protein); beta: SEQ ID NO: 147 (coding region) and 148 (protein); and gamma: SEQ ID NO: 149 (coding region) and 150 (protein)) were expressed in conjunction with the two subunits of the
20 *Klebsiella pneumoniae* glycerol dehydratase reactivase (large subunit, SEQ ID NO: 151 (coding region) and 152 (protein); and small subunit, SEQ ID NO: 153 (coding region) and 154 (protein)) to provide activity.

There are also reports in the literature of a B12-independent diol dehydratase from *Clostridium glycolicum* (Hartmanis *et al.* (1986) *Arch.*
25 *Biochem. Biophys.* 245:144-152). This enzyme has activity towards 2,3-butanediol, although this activity is less than 1% of the activity towards ethanediol, but the enzyme may be engineered to improve that activity. A better-characterized B12-independent dehydratase is the glycerol dehydratase from *Clostridium butyricum* (O'Brien *et al.* (2004)
30 *Biochemistry* 43:4635-4645), which has high activity towards 1,2-propanediol as well as glycerol. This enzyme uses S-adenosylmethionine as a source of adenosyl radical. There are no reports of activity towards

2,3-butanediol, but such activity, if not already present, may possibly be engineered.

The skilled person will appreciate that polypeptides having butanediol dehydrogenase activity isolated from a variety of sources will be useful in the present invention independent of sequence homology. As noted above a variety of diol and glycerol dehydratases have been described in the literature and will be suitable for use in the present invention. Accordingly, in one aspect of the invention preferred diol and glycerol dehydratase enzymes are those that have at least 80% – 85% identity to enzymes having the large, medium and small subunits, respectively of the sequences listed below:

- a) SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12;
- b) SEQ ID NO:93, SEQ ID NO:95, and SEQ ID NO:97;
- c) SEQ ID NO:99, SEQ ID NO:101, and SEQ ID NO:103;
- d) SEQ ID NO:105, SEQ ID NO:107, and SEQ ID NO:109;
- e) SEQ ID NO:135, SEQ ID NO:136, and SEQ ID NO:137;
- f) SEQ ID NO:138, SEQ ID NO:139, and SEQ ID NO:140;
- g) SEQ ID NO:146, SEQ ID NO:148, and SEQ ID NO:150;
- h) SEQ ID NO:141, SEQ ID NO:142, and SEQ ID NO:143; and
- i) SEQ ID NO:164, SEQ ID NO:165, and SEQ ID NO:166.

where at least 85% - 90% identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix, is most preferred.

Similarly preferred diol and glycerol dehydratase enzymes are those that have at least 80% – 85% identity to enzymes having the large, medium and small subunits, respectively of the sequences listed below:
Large subunit: SEQ ID NOs: 8, 99, 105, 135, 138, 141, 146, and 164;
Medium subunit: SEQ ID NOs: 10, 101, 107, 136, 139, 142, 148, and 165;
Small subunit: SEQ ID NOs: 12, 103, 109, 137, 140, 143, 150, and 166;
where at least 85% - 90% identity is more preferred and where at least 95% identity based on the Clustal W method of alignment using the default

parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix, is most preferred.

Additional diol and glycerol dehydratase enzymes that may be used in the biosynthetic pathway 3 of the present invention were identified
5 through a bioinformatics structure/function analysis that is described below and in Example 18.

(f) 2-butanone to 2-butanol:

This substrate to product conversion is the same as described above for Pathway 1.

10 Diol and Glycerol Dehydratases for Biosynthetic Pathway 3

Any enzyme that is a diol or glycerol dehydratase may be used in the present invention for the conversion of 2,3-butanediol to 2-butanone. A structure/function relationship for diol and glycerol dehydratases in the enzyme classes EC 4.2.1.28 and EC 4.2.1.30, respectively, was
15 established herein in Example 18. The function is provided by experimental data and the structure is provided by bioinformatics analysis. Eight diol and glycerol dehydratase enzymes with activities that have been experimentally demonstrated were analyzed. In this group (listed in Table
20 10), the *Klebsiella oxytoca* diol dehydratase and the *Klebsiella pneumoniae* glycerol dehydratase enzymes were both shown to convert 2,3-butanediol to 2-butanone (Bachovchin *et al.* (1977) *Biochemistry* 16:1082-1092 and Example 17 herein, respectively), while the activities of the additional six enzymes were demonstrated using their natural
25 substrates (references given in Table 10). This set of eight diol and glycerol dehydratases was analyzed using the hmmsearch algorithm of the HMMER software package (Janelia Farm Research Campus, Ashburn, VA). The Z parameter of the hmmsearch algorithm was set to 1 billion. The output of the HMMER analysis using a set of protein
30 sequences is a Profile Hidden Markov Model (Profile HMM). The theory behind Profile HMMs is described in Durbin *et al.*, Biological sequence analysis: probabilistic models of proteins and nucleic acids, Cambridge University Press, 1998; Krogh *et al.*, 1994; *J. Mol. Biol.* 235:1501–1531, incorporated herein by reference) that characterizes the set of proteins

based on the probability of each amino acid occurring at each position in the alignment of the proteins of the set.

Since the eight diol and glycerol dehydratases (diol/glycerol dehydratases) with experimentally verified function that were used for the analysis each have three subunits (large or alpha, medium or beta, and small or gamma), a separate Profile HMM was prepared for each subunit. The large subunit Profile HMM (Table 12) was built using proteins with SEQ ID NOs: 8, 99, 105, 135, 138, 141, 146, and 164 that are described in Tables 1 and 2. The medium subunit Profile HMM (Table 13) was built using proteins with SEQ ID NOs: 10, 101, 107, 136, 139, 142, 148, and 165 that are described in Tables 1 and 2. The small subunit Profile HMM (Table 14) was built using proteins with SEQ ID NOs: 12, 103, 109, 137, 140, 143, 150, and 166 that are described in Tables 1 and 2. References that provide the functional assay data are given in Table 10. The Profile HMM prepared for the large subunit gives a structural characterization for the functional large subunit of diol/glycerol dehydratases. Similarly the Profile HMMs for the medium and small subunits give structural characterizations for the functional medium and small subunits, respectively, of diol/glycerol dehydratases. Therefore any protein that has a significant match to either the large, medium, or small subunit Profile HMM is directly linked to the function of the subunit to which the profile was prepared. To be significant, the matching has an E-value of 0.01 or less, and further use of "match" is understood to be with this E-value criterion. Thus diol/glycerol dehydratase subunits that may be used in the present invention are proteins that match the Profile HMMs, that were prepared using the proteins with SEQ ID NOs listed above, with an E-value of 0.01 or less.

Proteins that are full length and have functional linkage to the large subunit of diol/glycerol dehydratases, through matching the large subunit Profile HMM, include but are not limited to, proteins with SEQ ID NOs; 93, 167, 170, 173, 176, 179, 182, 185, 188, 191, 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 130, 243, 254, 255, 256, 257, 258, and 259. Proteins that are full length and have functional linkage to the

medium subunit of diol/glycerol dehydratases, through matching the medium subunit Profile HMM, include but are not limited to, proteins with SEQ ID NOs; 95, 168, 171, 174, 177, 180, 183, 186, 189, 192, 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 244, 250, 252, 260, 261, 262, 263, 364, 265, 266, and 167. Proteins that are full length and have functional linkage to the small subunit of diol/glycerol dehydratases, through matching the small subunit Profile HMM, include but are not limited to, proteins with SEQ ID NOs; 97, 169, 172, 175, 178, 181, 184, 187, 190, 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 234, 236, 238, 240, 242, 245, 248, 249, 251, 253, 268, 270, 271, 272, 273, and 274. In addition, proteins that are fused full length large and medium subunits that have functional linkage to the large and medium subunits of diol/glycerol dehydratases, through matching the large and medium subunit Profiles HMM, include but are not limited to, proteins with SEQ ID NOs; 233, 235, 237, 239, 241, 246, and 247.

Since the Profile HMMs described above provide a structure/function relationship for diol/glycerol dehydratases, newly identified proteins that match these profiles may also be used in the present invention. In addition, diol/glycerol dehydratase subunit protein sequences that may be used in the present invention include proteins with amino acid changes that have minimal effects on subunit function, which are substantially similar to the sequences of the SEQ ID NOs listed above. It is well known in the art that substitution of a chemically equivalent amino acid at a given site which does not effect the functional properties of the encoded protein is common. For the purposes of the present invention substitutions providing substantially similar proteins are defined as exchanges within one of the following five groups:

1. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr (Pro, Gly);
2. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gln;
3. Polar, positively charged residues: His, Arg, Lys;
4. Large aliphatic, nonpolar residues: Met, Leu, Ile, Val (Cys); and

5. Large aromatic residues: Phe, Tyr, Trp.

Thus, substitutions of one amino acid for another in these groups can be expected to produce a functionally equivalent protein. In many cases, changes which result in alteration of the N-terminal and C-terminal portions of the protein would also not be expected to alter the activity of the protein.

Substantially similar proteins to those of SEQ IDs that match the Profile HMMs may be 90% or 95% identical in amino acid sequence to one of the matching proteins, and these may be used in the present invention.

One skilled in the art can readily identify a set of three subunits that may be used together to provide a functional diol/glycerol dehydratase. Particularly suitable is a combination of a large, medium and small subunit from the same strain of organism, whose coding regions are located near one another in the genome. These subunits would be most likely to form a natural diol or glycerol dehydratase. Many large, medium, and small subunits are grouped in this manner in Table 2. A combination of subunits from closely related strains or species is suitable for composing a diol dehydratase or a glycerol dehydratase. Any combination of subunits that catalyzes the conversion of 2,3-butanediol to 2-butanone may be used. Effective subunit combinations may readily be determined by one skilled in the art through amino acid sequence comparisons and/or functional assays.

Accordingly the invention provides diol and glycerol dehydratase enzymes having amino acid sequences comprising full length large, medium and small subunits that each give an E-value parameter of 0.01 or less when queried using a Profile Hidden Markov Model prepared using the large subunits of SEQ ID NOs: 8, 99, 105, 135, 138, 141, 146, and 164; the medium subunits of SEQ ID NOs: 10, 101, 107, 136, 139, 142, 148, and 165; and the small subunits of SEQ ID NOs: 12, 103, 109, 137, 140, 143, 150, and 166; each query being carried out using the hmmsearch algorithm wherein the Z parameter is set to 1 billion.

- Alternatively the invention provides diol and glycerol dehydratase enzymes having amino acid sequences identified by a process comprising a) generating a Profile Hidden Markov Model from the alignment of the amino acid sequences corresponding to the large, medium and small subunits of diol and glycerol dehydratase enzymes wherein;
- 5
- i) the large subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 99, 105, 135, 138, 141, 146, and 164;
- 10
- ii) the medium subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 101, 107, 136, 139, 142, 148, and 165; and
- iii) the small subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 103, 109, 137, 140, 143, 150, and 166;
- 15
- b) querying at least one public database of protein sequences containing sequences of diol and glycerol dehydratases with the Profile Hidden Markov Model of (a) using the hmmsearch algorithm wherein the Z parameter is set to 1 billion and the E-value parameter is set to 0.01, to identify a first data set of diol and glycerol dehydratase amino acid sequences; and
- 20
- c) removing any partial sequences from the first data set of (b) to generate a second data set of diol and glycerol dehydratase amino acid sequences, wherein diol dehydratase and the glycerol dehydratase enzymes are identified.
- 25

With respect to large subunits of the diol and glycerol dehydratases of the invention the enzymes may comprise a large subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 93, 99, 105, 135, 138, 141, 146, 164, 167, 170, 173, 176, 179, 182, 185, 188, 191, 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 130, 243, 254, 255, 256, 257, 258 and 259, based on the Clustal W method of

30

alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

With respect to the medium subunits of the diol and glycerol dehydratases of the invention the enzymes may comprise a medium subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 95, 101, 107, 136, 139, 142, 148, 165, 168, 171, 174, 177, 180, 183, 186, 189, 192, 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 244, 250, 252, 260, 261, 262, 263, 364, 265, 266, and 167 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

With respect to the small subunits of the diol and glycerol dehydratases of the invention the enzymes may comprise a medium subunit comprising a small subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 97, 103, 109, 137, 140, 143, 150, 166, 169, 172, 175, 178, 181, 184, 187, 190, 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 234, 236, 238, 240, 242, 245, 248, 249, 251, 253, 268, 270, 271, 272, 273, and 274, based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

Alternatively the diol dehydratase or glycerol dehydratase may comprise fused large, medium and small subunits comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 233, 235, 237, 239, 241, 246, and 247, based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

Alternatively the diol dehydratase or glycerol dehydratase enzymes may comprise a fused large, medium and small subunits and has at least 95% identity to an amino acid sequence comprising all three of the amino

acid sequences encoding large, medium and small subunits, selected from the group consisting of:

- a) SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12;
- b) SEQ ID NO:93, SEQ ID NO:95, and SEQ ID NO:97;
- 5 c) SEQ ID NO:99, SEQ ID NO:101, and SEQ ID NO:103;
- d) SEQ ID NO:105, SEQ ID NO:107, and SEQ ID NO:109;
- e) SEQ ID NO:135, SEQ ID NO:136, and SEQ ID NO:137;
- f) SEQ ID NO:138, SEQ ID NO:139, and SEQ ID NO:140;
- g) SEQ ID NO:146, SEQ ID NO:148, and SEQ ID NO:150;
- 10 h) SEQ ID NO:141, SEQ ID NO:142, and SEQ ID NO:143; and
- i) SEQ ID NO:164, SEQ ID NO:165, and SEQ ID NO:166;

based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

15 Pathway 4:

(a) pyruvate to alpha-acetolactate:

This substrate to product conversion is the same as described above for Pathway 1.

(k) alpha-acetolactate to 2,3-dihydroxy-2-methylbutanoic acid:

20 The substrate to product conversion of acetolactate (I) to 2,3-dihydroxy-2-methylbutanoic acid (IX) is not known in the art. However, the product of this conversion has been reported as a component of fermentation broths (Ziadi et al. (1973) *Comptes Rendus des Seances de l'Academie des Sciences, Serie D: Sciences Naturelles* 276:965-8), but

25 the mechanism of formation is unknown. The likely mechanism of formation is reduction of acetolactate with NADH or NADPH as the electron donor. To utilize this pathway for production of 2-butanol, an enzyme catalyzing this reaction needs to be identified or engineered. However, the precedent for enzymatic reduction of ketones to alcohols is

30 well established.

(l) 2,3-dihydroxy-2-methylbutanoic acid to 2-hydroxy-2-methyl-3-phosphobutanoic acid:

There are no enzymes known that catalyze the substrate to product conversion of 2,3-dihydroxy-2-methylbutanoic acid (IX) to 2-hydroxy-2-methyl-3-phosphobutanoic acid (X). However, there are a large number of kinases in Nature that possess varying specificity. It is therefore likely that an enzyme could be isolated or engineered with this activity.

(m) 2-hydroxy-2-methyl-3-phosphobutanoic acid to 2-butanone:

There are no known enzymes that catalyze the substrate to product conversion of 2-hydroxy-2-methyl-3-phosphobutanoic acid (X) to 2-butanone (V). The combination of this reaction with the previous one is very similar to the multi-step reaction catalyzed by mevalonate-5-pyrophosphate (M5PP) decarboxylase, which consists of initial phosphorylation of M5PP to 3-phosphomevalonate-5-PP, followed by decarboxylation-dependent elimination of phosphate (Alvear *et al.* (1982) *Biochemistry* 21:4646-4650).

(f) 2-butanone to 2-butanol:

This substrate to product conversion is the same as described above for Pathway 1.

Thus, in providing multiple recombinant pathways from pyruvate to 2-butanol, there exists a number of choices to fulfill the individual conversion steps, and the person of skill in the art will be able to utilize publicly available sequences and sequences disclosed herein to construct the relevant pathways. A listing of a representative number of genes known in the art and useful in the construction of 2-butanol biosynthetic pathways is given above in Tables 1 and 2.

Microbial Hosts for 2-Butanol and 2-Butanone Production

Microbial hosts for 2-butanol or 2-butanone production may be selected from bacteria, cyanobacteria, filamentous fungi and yeasts. The microbial host used for 2-butanol or 2-butanone production should be tolerant to the product produced, so that the yield is not limited by toxicity of the product to the host. The selection of a microbial host for 2-butanol production is described in detail below. The same criteria apply to the selection of a host for 2-butanone production.

Microbes that are metabolically active at high titer levels of 2-butanol are not well known in the art. Although butanol-tolerant mutants have been isolated from solventogenic *Clostridia*, little information is available concerning the butanol tolerance of other potentially useful bacterial strains. Most of the studies on the comparison of alcohol tolerance in bacteria suggest that butanol is more toxic than ethanol (de Cavalho et al., *Microsc. Res. Tech.* 64:215-22 (2004) and Kabelitz et al., *FEMS Microbiol. Lett.* 220:223-227 (2003)). Tomas et al. (*J. Bacteriol.* 186:2006-2018 (2004)) report that the yield of 1-butanol during fermentation in *Clostridium acetobutylicum* may be limited by butanol toxicity. The primary effect of 1-butanol on *Clostridium acetobutylicum* is disruption of membrane functions (Hermann et al., *Appl. Environ. Microbiol.* 50:1238-1243 (1985)).

The microbial hosts selected for the production of 2-butanol should be tolerant to 2-butanol and should be able to convert carbohydrates to 2-butanol using the introduced biosynthetic pathway. The criteria for selection of suitable microbial hosts include the following: intrinsic tolerance to 2-butanol, high rate of carbohydrate utilization, availability of genetic tools for gene manipulation, and the ability to generate stable chromosomal alterations.

Suitable host strains with a tolerance for 2-butanol may be identified by screening based on the intrinsic tolerance of the strain. The intrinsic tolerance of microbes to 2-butanol may be measured by determining the concentration of 2-butanol that is responsible for 50% inhibition of the growth rate (IC₅₀) when grown in a minimal medium. The IC₅₀ values may be determined using methods known in the art. For example, the microbes of interest may be grown in the presence of various amounts of 2-butanol and the growth rate monitored by measuring the optical density at 600 nanometers. The doubling time may be calculated from the logarithmic part of the growth curve and used as a measure of the growth rate. The concentration of 2-butanol that produces 50% inhibition of growth may be determined from a graph of the percent inhibition of growth versus the 2-butanol concentration. Preferably, the host strain should

have an IC50 for 2-butanol of greater than about 0.5%. More suitable is a host strain with an IC50 for 2-butanol that is greater than about 1.5%. Particularly suitable is a host strain with an IC50 for 2-butanol that is greater than about 2.5%.

5 The microbial host for 2-butanol production should also utilize glucose and/or other carbohydrates at a high rate. Most microbes are capable of utilizing carbohydrates. However, certain environmental microbes cannot efficiently use carbohydrates, and therefore would not be suitable hosts.

10 The ability to genetically modify the host is essential for the production of any recombinant microorganism. Modes of gene transfer technology that may be used include by electroporation, conjugation, transduction or natural transformation. A broad range of host conjugative plasmids and drug resistance markers are available. The cloning vectors
15 used with an organism are tailored to the host organism based on the nature of antibiotic resistance markers that can function in that host.

 The microbial host also may be manipulated in order to inactivate competing pathways for carbon flow by inactivating various genes. This requires the availability of either transposons or chromosomal integration
20 vectors to direct inactivation. Additionally, production hosts that are amenable to chemical mutagenesis may undergo improvements in intrinsic 2-butanol tolerance through chemical mutagenesis and mutant screening.

 Based on the criteria described above, suitable microbial hosts for
25 the production of 2-butanol and 2-butanone include, but are not limited to, members of the genera *Clostridium*, *Zymomonas*, *Escherichia*, *Salmonella*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Alcaligenes*, *Klebsiella*, *Paenibacillus*, *Arthrobacter*, *Corynebacterium*, *Brevibacterium*, *Pichia*, *Candida*,
30 *Hansenula* and *Saccharomyces*. Preferred hosts include: *Escherichia coli*, *Alcaligenes eutrophus*, *Bacillus licheniformis*, *Paenibacillus macerans*, *Rhodococcus erythropolis*, *Pseudomonas putida*, *Lactobacillus plantarum*, *Enterococcus faecium*, *Enterococcus gallinarum*,

Enterococcus faecalis, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Bacillus subtilis* and *Saccharomyces cerevisiae*.

Construction of Production Host

Recombinant organisms containing the necessary genes that
5 encode the enzymatic pathway for the conversion of a fermentable carbon
substrate to 2-butanol or 2-butanone may be constructed using techniques
well known in the art. In the present invention, genes encoding the
enzymes of the 2-butanol biosynthetic Pathway 3: acetolactate synthase,
acetolactate decarboxylase, butanediol dehydrogenase, butanediol
10 dehydratase, and butanol dehydrogenase; or 2-butanone biosynthetic
Pathway 3 omitting the butanol dehydrogenase, may be isolated from
various sources, as described above.

Methods of obtaining desired genes from a bacterial genome are
common and well known in the art of molecular biology. For example, if
15 the sequence of the gene is known, primers may be designed and the
desired sequence amplified using standard primer-directed amplification
methods such as polymerase chain reaction (U.S. Patent No. 4,683,202)
to obtain amounts of DNA suitable for cloning into expression vectors. If a
gene that is heterologous to a known sequence is to be isolated, suitable
20 genomic libraries may be created by restriction endonuclease digestion
and may be screened with probes having complementary sequence to the
desired gene sequence. Once the sequence is isolated, the DNA may be
amplified using standard primer-directed amplification methods such as
polymerase chain reaction (U.S. 4,683,202) to obtain amounts of DNA
25 suitable for cloning into expression vectors, which are then transformed
into appropriate host cells.

In addition, given the amino acid sequence of a protein with desired
enzymatic activity, the coding sequence may be ascertained by reverse
translating the protein sequence. A DNA fragment containing the coding
30 sequence may be prepared synthetically and cloned into an expression
vector, then transformed into the desired host cell.

In preparing a synthetic DNA fragment containing a coding
sequence, this sequence may be optimized for expression in the target

host cell. Tools for codon optimization for expression in a heterologous host are readily available. Some tools for codon optimization are available based on the GC content of the host organism. The GC contents of some exemplary microbial hosts are given Table 3.

5.

Table 3
GC Contents of Microbial Hosts

Strain	% GC
<i>B. licheniformis</i>	46
<i>B. subtilis</i>	42
<i>C. acetobutylicum</i>	37
<i>E. coli</i>	50
<i>P. putida</i>	61
<i>A. eutrophus</i>	61
<i>Paenibacillus macerans</i>	51
<i>Rhodococcus erythropolis</i>	62
<i>Brevibacillus</i>	50
<i>Paenibacillus polymyxa</i>	50

Once the relevant pathway genes are identified and isolated they may be transformed into suitable expression hosts by means well known in the art. Vectors useful for the transformation of a variety of host cells are common and commercially available from companies such as EPICENTRE[®] (Madison, WI), Invitrogen Corp. (Carlsbad, CA), Stratagene (La Jolla, CA), and New England Biolabs, Inc. (Beverly, MA). Typically the vector contains a selectable marker and sequences allowing autonomous replication or chromosomal integration in the desired host. In addition, suitable vectors comprise a promoter region which harbors transcriptional initiation controls and a transcriptional termination control region, between which a coding region DNA fragment may be inserted, to provide expression of the inserted coding region. Both control regions may be derived from genes homologous to the transformed host cell, although it is to be understood that such control regions may also be derived from

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genes that are not native to the specific species chosen as a production host.

Initiation control regions or promoters, which are useful to drive expression of the relevant pathway coding regions in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genetic elements is suitable for the present invention including, but not limited to, promoters derived from the following genes: *CYC1*, *HIS3*, *GAL1*, *GAL10*, *ADH1*, *PGK*, *PHO5*, *GAPDH*, *ADC1*, *TRP1*, *URA3*, *LEU2*, *ENO*, *TPI*, *CUP1*, *FBA*, *GPD*, and *GPM* (useful for expression in *Saccharomyces*); *AOX1* (useful for expression in *Pichia*); as well as the *lac*, *ara*, *tet*, *trp*, *IP_L*, *IP_R*, *T7*, *tac*, and *trc* promoters (useful for expression in *Escherichia coli*, *Alcaligenes*, and *Pseudomonas*); the *amy*, *apr*, and *npr* promoters, and various phage promoters useful for expression in *Bacillus subtilis*, *Bacillus licheniformis*, and *Paenibacillus macerans*; *nisA* (useful for expression Gram-positive bacteria, Eichenbaum et al. *Appl. Environ. Microbiol.* 64(8):2763-2769 (1998)); and the synthetic P11 promoter (useful for expression in *Lactobacillus plantarum*, Rud et al., *Microbiology* 152:1011-1019 (2006)).

Termination control regions may also be derived from various genes native to the preferred hosts. Optionally, a termination site may be unnecessary, however, it is most preferred if included.

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)). Several plasmid derivatives of broad-host-range Inc P4 plasmid RSF1010 are also available with promoters that can function in a range of Gram-negative bacteria. Plasmid pAYC36 and pAYC37, have active promoters along with multiple cloning sites to allow for heterologous gene expression in Gram-negative bacteria.

Chromosomal gene replacement tools are also widely available. For example, a thermosensitive variant of the broad-host-range replicon pWV101 has been modified to construct a plasmid pVE6002 which can be used to effect gene replacement in a range of Gram-positive bacteria
5 (Maguin et al., *J. Bacteriol.* 174(17):5633-5638 (1992)). Additionally, in vitro transposomes are available from commercial sources such as EPICENTRE[®] to create random mutations in a variety of genomes.

The expression of a 2-butanone biosynthetic pathway in various preferred microbial hosts is described in more detail below. For the
10 expression of a 2-butanone biosynthetic pathway, the same description applies, but the final substrate to product conversion of 2-butanone to 2-butanol is omitted.

Expression of a 2-butanol or 2-butanone biosynthetic pathway in *E. coli*

15 Vectors useful for the transformation of *E. coli* are common and commercially available from the companies listed above. For example, the genes of a 2-butanol biosynthetic pathway may be isolated from various sources, as described above, cloned onto a modified pUC19 vector and transformed into *E. coli* NM522, as described in Examples 6
20 and 7. Alternatively, the genes encoding a 2-butanol biosynthetic pathway may be divided into multiple operons, cloned onto expression vectors, and transformed into various *E. coli* strains, as described in Examples 9, 10, and 11. The 2-butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

25 Expression of a 2-butanol or 2-butanone biosynthetic pathway in *Rhodococcus erythropolis*

A series of *E. coli*-*Rhodococcus* shuttle vectors are available for expression in *R. erythropolis*, including, but not limited to pRhBR17 and pDA71 (Kostichka et al., *Appl. Microbiol. Biotechnol.* 62:61-68 (2003)).
30 Additionally, a series of promoters are available for heterologous gene expression in *R. erythropolis* (see for example Nakashima et al., *Appl. Environ. Microbiol.* 70:5557-5568 (2004), and Tao et al., *Appl. Microbiol.*

Biotechnol. 2005, DOI 10.1007/s00253-005-0064). Targeted gene disruptions in chromosomal genes of *R. erythropolis* may be created using the methods described by Tao et al., *supra*, and Brans et al. (*Appl. Environ. Microbiol.* 66: 2029-2036 (2000)).

5 The heterologous genes required for the production of 2-butanol, as described above, may be cloned initially in pDA71 or pRhBR71 and transformed into *E. coli*. The vectors may then be transformed into *R. erythropolis* by electroporation, as described by Kostichka et al., *supra*. The recombinants may be grown in synthetic medium containing glucose
10 and the production of 2-butanol can be followed using fermentation methods known in the art. The 2-butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

Expression of a 2-butanol or 2-butanone biosynthetic pathway in *B. Subtilis*

15 Methods for gene expression and creation of mutations in *B. subtilis* are also well known in the art. For example, the genes of a 2-butanol biosynthetic pathway may be isolated from various sources, as described above, cloned into a modified *E. coli-Bacillus* shuttle vector and transformed into *Bacillus subtilis* BE1010, as described in Example 8, The
20 desired genes may be cloned into a *Bacillus* expression vector and transformed into a strain to make a production host. Alternatively, the genes may be integrated into the *Bacillus* chromosome using conditional replicons or suicide vectors that are known to one skilled in the art. For example, the *Bacillus* Genetic Stock Center carries numerous integration
25 vectors. The 2-butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

Expression of a 2-butanol or 2-butanone biosynthetic pathway in *B. licheniformis*

30 Most of the plasmids and shuttle vectors that replicate in *B. subtilis* may be used to transform *B. licheniformis* by either protoplast transformation or electroporation. The genes required for the production of 2-butanol may be cloned in plasmids pBE20 or pBE60 derivatives (Nagarajan et al., *Gene* 114:121-126 (1992)). Methods to transform *B.*

licheniformis are known in the art (for example see Fleming et al. *Appl. Environ. Microbiol.*, 61(11):3775-3780 (1995)). The plasmids constructed for expression in *B. subtilis* may be transformed into *B. licheniformis* to produce a recombinant microbial host that produces 2-butanol. The 2-
5 butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

Expression of a 2-butanol or 2-butanone biosynthetic pathway in
Paenibacillus macerans

Plasmids may be constructed as described above for expression in
10 *B. subtilis* and used to transform *Paenibacillus macerans* by protoplast transformation to produce a recombinant microbial host that produces 2-butanol. The 2-butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

Expression of a 2-butanol or 2-butanone biosynthetic pathway in
15 *Alcaligenes (Ralstonia) eutrophus*

Methods for gene expression and creation of mutations in
Alcaligenes eutrophus are known in the art (see for example Taghavi et al., *Appl. Environ. Microbiol.*, 60(10):3585-3591 (1994)). The genes for a 2-butanol biosynthetic pathway may be cloned in any of the broad host
20 range vectors described above, and electroporated into *Alcaligenes eutrophus* to generate recombinants that produce 2-butanol. The poly(hydroxybutyrate) pathway in *Alcaligenes* has been described in detail, a variety of genetic techniques to modify the *Alcaligenes eutrophus* genome are known, and those tools can be applied for engineering a 2-
25 butanol biosynthetic pathway. The 2-butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

Expression of a 2-butanol or 2-butanone biosynthetic pathway in
Pseudomonas putida

Methods for gene expression in *Pseudomonas putida* are known in
30 the art (see for example Ben-Bassat et al., U.S. Patent No. 6,586,229, which is incorporated herein by reference). The genes of a 2-butanol biosynthetic pathway may be inserted into pPCU18, and this ligated DNA may be electroporated into electrocompetent *Pseudomonas putida* DOT-

T1 C5aAR1 cells to generate recombinants that produce 2-butanol. The 2-butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

5 Expression of a 2-butanol or 2-butanone biosynthetic pathway in
Lactobacillus plantarum

The *Lactobacillus* genus belongs to the *Lactobacillales* family and many plasmids and vectors used in the transformation of *Bacillus subtilis* and *Streptococcus* may be used for *Lactobacillus*. Non-limiting examples of suitable vectors include pAM β 1 and derivatives thereof (Renault et al.,
 10 *Gene* 183:175-182 (1996); and O'Sullivan et al., *Gene* 137:227-231 (1993)); pMBB1 and pHW800, a derivative of pMBB1 (Wyckoff et al. *Appl. Environ. Microbiol.* 62:1481-1486 (1996)); pMG1, a conjugative plasmid (Tanimoto et al., *J. Bacteriol.* 184:5800-5804 (2002)); pNZ9520 (Kleerebezem et al., *Appl. Environ. Microbiol.* 63:4581-4584 (1997));
 15 pAM401 (Fujimoto et al., *Appl. Environ. Microbiol.* 67:1262-1267 (2001)); and pAT392 (Arthur et al., *Antimicrob. Agents Chemother.* 38:1899-1903 (1994)). Several plasmids from *Lactobacillus plantarum* have also been reported (van Kranenburg et al., *Appl. Environ. Microbiol.* 71(3):1223-1230 (2005)).

20 The various genes for a 2-butanol biosynthetic pathway may be assembled into any suitable vector, such as those described above. The codons can be optimized for expression based on the codon index deduced from the genome sequences of *Lactobacillus plantarum* or *Lactobacillus arizonensis*. The plasmids may be introduced into the host
 25 cell using methods known in the art, such as electroporation (Cruz-Rodz et al. *Molecular Genetics and Genomics* 224:1252-154 (1990), Bringel, et al. *Appl. Microbiol. Biotechnol.* 33: 664-670 (1990), Alegre et al., *FEMS Microbiology letters* 241:73-77 (2004)), and conjugation (Shrago et al., *Appl. Environ. Microbiol.* 52:574-576 (1986)). The 2-butanol biosynthetic
 30 pathway genes can also be integrated into the chromosome of *Lactobacillus* using integration vectors (Hols et al., *Appl. Environ. Microbiol.* 60:1401-1403 (1990), Jang et al., *Micro. Lett.* 24:191-195

(2003)). The 2-butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

Expression of a 2-butanol or 2-butanone biosynthetic pathway in
Enterococcus faecium, *Enterococcus gallinarum*, and *Enterococcus*
5 *faecalis*

The *Enterococcus* genus belongs to the *Lactobacillales* family and many plasmids and vectors used in the transformation of *Lactobacillus*, *Bacillus subtilis*, and *Streptococcus*, described above, may be used for *Enterococcus*. Expression vectors for *E. faecalis* using the *nisA* gene from
10 *Lactococcus* may also be used (Eichenbaum et al., *Appl. Environ. Microbiol.* 64:2763-2769 (1998). Additionally, vectors for gene replacement in the *E. faecium* chromosome may be used (Nallaapareddy et al., *Appl. Environ. Microbiol.* 72:334-345 (2006)).

The various genes for a 2-butanol biosynthetic pathway may be
15 assembled into any suitable vector, such as those described above. The codons can be optimized for expression based on the codon index deduced from the genome sequences of *Enterococcus faecalis* or *Enterococcus faecium*. The plasmids may be introduced into the host cell using methods known in the art, such as electroporation, as described by
20 Cruz-Rodz et al. (*Molecular Genetics and Genomics* 224:1252-154 (1990)) or conjugation, as described by Tanimoto et al. (*J. Bacteriol.* 184:5800-5804 (2002)) and Grohamann et al. (*Microbiol. Mol. Biol. Rev.* 67:277-301 (2003)). The 2-butanone biosynthesis pathway may be similarly expressed, omitting the butanol dehydrogenase.

25 Expression of a 2-butanol or 2-butanone biosynthetic pathway in
Pediococcus pentosaceus and *Pediococcus acidilactici*,

The *Pediococcus* genus belongs to the *Lactobacillales* family and many plasmids and vectors used in the transformation of *Bacillus subtilis* and *Streptococcus*, described above, may be used for *Pediococcus*. A
30 non-limiting example of a suitable vector is pHPS9 (Bukhtiyarova et al. *Appl. Environ. Microbiol.* 60:3405-3408 (1994)). Several plasmids from *Pediococcus* have also been reported (Alegre et al., *FEMS Microbiol. Lett.*

250:151-156 (2005); Shareck et al. *Crit. Rev Biotechnol.* 24:155-208 (2004)).

The genes for a 2-butanol biosynthetic pathway may be assembled into any suitable vector, such as those described above. The codons can
5 be optimized for expression based on the codon index deduced from the genome sequence of *Pediococcus pentosaceus*. The plasmids may be introduced into the host cell using methods known in the art, such as electroporation (see for example, Osmanagaoglu et al., *J. Basic Microbiol.* 40:233-241 (2000); Alegre et al., *FEMS Microbiol. Lett.* 250:151-156
10 (2005)) and conjugation (Gonzalez and Kunka, *Appl. Environ. Microbiol.* 46:81-89 (1983)). The 2-butanol biosynthetic pathway genes can also be integrated into the chromosome of *Pediococcus* using integration vectors (Davidson et al. *Antonie van Leeuwenhoek* 70:161-183 (1996)). The 2-
butanone biosynthesis pathway may be similarly expressed, omitting the
15 butanol dehydrogenase.

Fermentation Media

Fermentation media in the present invention must contain suitable carbon substrates. Suitable substrates may include but are not limited to monosaccharides such as glucose and fructose, oligosaccharides such as
20 lactose or sucrose, polysaccharides such as starch or cellulose or mixtures thereof and unpurified mixtures from renewable feedstocks such as cheese whey permeate, cornsteep liquor, sugar beet molasses, and barley malt. Additionally the carbon substrate may also be one-carbon substrates such as carbon dioxide, or methanol for which metabolic
25 conversion into key biochemical intermediates has been demonstrated. In addition to one and two carbon substrates, methylotrophic organisms are also known to utilize a number of other carbon containing compounds such as methylamine, glucosamine and a variety of amino acids for metabolic activity. For example, methylotrophic yeasts are known to
30 utilize the carbon from methylamine to form trehalose or glycerol (Bellion et al., *Microb. Growth C1 Compd.*, [Int. Symp.], 7th (1993), 415-32, Editor(s): Murrell, J. Collin; Kelly, Don P. Publisher: Intercept, Andover, UK). Similarly, various species of *Candida* will metabolize alanine or oleic

acid (Sulter et al., *Arch. Microbiol.* 153:485-489 (1990)). Hence it is contemplated that the source of carbon utilized in the present invention may encompass a wide variety of carbon containing substrates and will only be limited by the choice of organism.

5 Although it is contemplated that all of the above mentioned carbon substrates and mixtures thereof are suitable in the present invention, preferred carbon substrates are glucose, fructose, and sucrose, as well as mixtures of any of these sugars. Sucrose may be obtained from feedstocks such as sugar cane, sugar beets, cassava, and sweet
10 sorghum. Glucose and dextrose may be obtained through saccharification of starch based feedstocks including grains such as corn, wheat, rye, barley, and oats.

 In addition, fermentable sugars may be obtained from cellulosic and lignocellulosic biomass through processes of pretreatment and
15 saccharification, as described, for example, in co-owned and co-pending US patent application US20070031918A1, which is herein incorporated by reference. Biomass refers to any cellulosic or lignocellulosic material and includes materials comprising cellulose, and optionally further comprising hemicellulose, lignin, starch, oligosaccharides and/or monosaccharides.
20 Biomass may also comprise additional components, such as protein and/or lipid. Biomass may be derived from a single source, or biomass can comprise a mixture derived from more than one source; for example, biomass could comprise a mixture of corn cobs and corn stover, or a mixture of grass and leaves. Biomass includes, but is not limited to,
25 bioenergy crops, agricultural residues, municipal solid waste, industrial solid waste, sludge from paper manufacture, yard waste, wood and forestry waste. Examples of biomass include, but are not limited to, corn grain, corn cobs, crop residues such as corn husks, corn stover, grasses, wheat, wheat straw, barley, barley straw, hay, rice straw, switchgrass,
30 waste paper, sugar cane bagasse, sorghum, soy, components obtained from milling of grains, trees, branches, roots, leaves, wood chips, sawdust, shrubs and bushes, vegetables, fruits, flowers and animal manure.

In addition to an appropriate carbon source, fermentation media must contain suitable minerals, salts, cofactors, buffers and other components, known to those skilled in the art, suitable for the growth of the cultures and promotion of an enzymatic pathway necessary for 2-
5 butanol or 2-butanone production.

Culture Conditions

Typically cells are grown at a temperature in the range of about 25 °C to about 40 °C in an appropriate medium. Suitable growth media in the present invention are common commercially prepared media such as
10 Luria Bertani (LB) broth, Sabouraud Dextrose (SD) broth or Yeast Medium (YM) broth. Other defined or synthetic growth media may also be used, and the appropriate medium for growth of the particular microorganism will be known by one skilled in the art of microbiology or fermentation science. The use of agents known to modulate catabolite repression directly or
15 indirectly, e.g., cyclic adenosine 2':3'-monophosphate, may also be incorporated into the fermentation medium.

Suitable pH ranges for the fermentation are between pH 5.0 to pH 9.0, where pH 6.0 to pH 8.0 is preferred as the initial condition.

Fermentations may be performed under aerobic or anaerobic
20 conditions, where anaerobic or microaerobic conditions are preferred.

Industrial Batch and Continuous Fermentations

The present process employs a batch method of fermentation. A classical batch fermentation is a closed system where the composition of the medium is set at the beginning of the fermentation and not subject to
25 artificial alterations during the fermentation. Thus, at the beginning of the fermentation the medium is inoculated with the desired organism or organisms, and fermentation is permitted to occur without adding anything to the system. Typically, however, a "batch" fermentation is batch with respect to the addition of carbon source and attempts are often made at
30 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 fermentation is stopped. 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 generally are responsible for the bulk of production of end product or intermediate.

- 5 A variation on the standard batch system is the fed-batch system. Fed-batch fermentation processes are also suitable in the present invention and comprise a typical batch system with the exception that the substrate is added in increments as the fermentation progresses. Fed-batch systems are useful when catabolite repression is apt to inhibit
- 10 the metabolism of the cells and where it is desirable to have limited amounts of substrate in the media. 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, dissolved oxygen and the partial pressure of waste gases such as CO₂. Batch and
- 15 fed-batch fermentations are common and well known in the art and examples may be found in Thomas D. Brock in *Biotechnology: A Textbook of Industrial Microbiology*, Second Edition (1989) Sinauer Associates, Inc., Sunderland, MA., or Deshpande, Mukund V., *Appl. Biochem. Biotechnol.*, 36:227, (1992), herein incorporated by reference.
- 20 Although the present invention is performed in batch mode it is contemplated that the method would be adaptable to continuous fermentation methods. Continuous fermentation is an open system where a defined fermentation medium is added continuously to a bioreactor and an equal amount of conditioned media is removed simultaneously for
- 25 processing. Continuous fermentation generally maintains the cultures at a constant high density.
- Continuous fermentation 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
- 30 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 the turbidity of the culture medium, is kept constant. Continuous

systems strive to maintain steady state growth conditions and thus the cell loss due to the medium being drawn off must be balanced against the cell growth rate in the fermentation. Methods of modulating nutrients and growth factors for continuous fermentation processes as well as
5 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*.

It is contemplated that the present invention may be practiced using either batch, fed-batch or continuous processes and that any known mode
10 of fermentation would be suitable. Additionally, it is contemplated that cells may be immobilized on a substrate as whole cell catalysts and subjected to fermentation conditions for 2-butanol or 2-butanone production.

Methods for 2-Butanol and 2-Butanone Isolation from the Fermentation
15 Medium

The bioproducted 2-butanol may be isolated from the fermentation medium using methods known in the art for ABE fermentations (see for example, Durre, *Appl. Microbiol. Biotechnol.* 49:639-648 (1998), Groot et al., *Process Biochem.* 27:61-75 (1992), and references therein). For
20 example, solids may be removed from the fermentation medium by centrifugation, filtration, decantation, or the like. Then, the 2-butanol may be isolated from the fermentation medium using methods such as distillation, azeotropic distillation, liquid-liquid extraction, adsorption, gas stripping, membrane evaporation, or pervaporation. These same methods
25 may be adapted to isolate bioproducted 2-butanone from the fermentation medium.

EXAMPLES

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating a preferred
30 embodiment of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and, without departing from the spirit and scope thereof, can make various changes

and modifications of the invention to adapt it to various uses and conditions.

GENERAL METHODS

Standard recombinant DNA and molecular cloning techniques
 5 described in the Examples are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, (1989) (Maniatis) and by T. J. Silhavy, M. L. Bennis, and L. W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor
 10 Laboratory Press, Cold Spring Harbor, N.Y. (1984) and by Ausubel, F. M. *et al.*, *Current Protocols in Molecular Biology*, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987).

Materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Techniques suitable for use in
 15 the following Examples may be found as set out in *Manual of Methods for General Bacteriology* (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds), American Society for Microbiology, Washington, DC. (1994)) or by Thomas D. Brock in *Biotechnology: A Textbook of Industrial*
 20 *Microbiology*, Second Edition, Sinauer Associates, Inc., Sunderland, MA (1989). All reagents, restriction enzymes and materials described for the growth and maintenance of bacterial cells were obtained from Aldrich Chemicals (Milwaukee, WI), BD Diagnostic Systems (Sparks, MD), Life Technologies (Rockville, MD), or Sigma Chemical Company (St. Louis,
 25 MO) unless otherwise specified. Bacterial strains are obtained from the American Type Culture Collection (ATCC, Manassas, VA) unless otherwise noted.

Oligonucleotide primers described in the following Examples are given in Table 4. All oligonucleotide primers were synthesized by Sigma-Genosys

30 (Woodlands, TX). Table 4

Cloning and Screening Primers

Gene	Primer Name	Sequence	SEQ ID NO:	Description
<i>budB</i>	B1	CACCATGGACAAACAGTA	15	<i>budB</i>

		TCCGGTACGCC		forward
<i>budB</i>	B2	CGAAGGGCGATAGCTTTA CCAATCC	16	<i>budB</i> reverse
<i>budA</i>	B3	CACCATGAATCATTCTGC TGAATGCACCTGCG	17	<i>budA</i> forward
<i>budA</i>	B4	GATACTGTTTGTCCATGT GACC	18	<i>budA</i> reverse
<i>budC</i>	B5	CACCATGAAAAAAGTCGC ACTTGTTACC	19	<i>budC</i> forward
<i>budC</i>	B6	TTAGTTAAATACCAT	20	<i>budC</i> reverse
<i>pddA</i>	B7	CACCATGAGATCGA AAAGATTG	21	<i>pddABC</i> forward
<i>pddC</i>	B8	CTTAGAGAAGTTAATCGT CGCC	22	<i>pddABC</i> reverse
<i>sadh</i>	B9	CACCATGAAAGCCCTCCA GTACACC	23	<i>sadh</i> forward
<i>sadh</i>	B10	CGTCGTGTCATGCCCGG G	24	<i>sadh</i> reverse
<i>budA</i>	B11	GATCGAATTCGTTTAAACT TAGTTTTCTACCGCACG	25	<i>budABC</i> forward
<i>budC</i>	B12	GATCGCATGCAAGCTTTC ATATAGTCGGAATTCC	26	<i>budABC</i> reverse
<i>pddA</i>	B13	GATCGAATTCGTTTAAACA AAGGAGGTCTGATTCATG AGATCG	27	<i>pddABC</i> forward
<i>pddC</i>	B14	GATCGGATTCTTAATCGT CGCC	28	<i>pddABC</i> reverse
<i>sadh</i>	B15	GATCGGATCCAAAGGAGG TCGGGCGCATGAAAGCC C	29	<i>sadh</i> forward
<i>sadh</i>	B16	GATCTCTAGAAAGCTTTC AGCCCGGGACGACC	30	<i>sadh</i> reverse
--	BenF	ACTTTCTTTCGCCTGTTTC AC	31	--
--	BenBPR	CATGAAGCTTGTTTAAACT CGGTGACCTTGAAAATAA TGAAAACCTTATATTGTTT GAAAATAATGAAAACCTAT ATTG	32	--
<i>budAB</i>	BABC F	GAGCTCGAATTCAAAGGA GGAAGTGTATATGAATCA TTC	33	<i>budAB</i> forward
<i>budAB</i>	BAB R	GGATCCTCTAGAATTAGT TAAATACCATCCCGCCG	34	<i>budAB</i> reverse
<i>budC</i>	BC Spe F	ACTAGTAAAGGAGGAAAG AGTATGAAGAAGGTCGCA CT	40	<i>budC</i> forward
<i>budC</i>	BC Xba R	TCTAGAAAGCAGGGGCAA GCCATGTC	41	<i>budC</i> reverse
<i>pddAB</i> C- ddrAB	DDo For	AAGCTTAAAGGAGGCTGA TTCATGAGATCGAAAAGA TT	44	<i>pddABC-ddrAB</i> forward
<i>pddAB</i> C- ddrAB	DDo Rev	TCTAGATTATTCATCCTGC TGTTCTCC	45	<i>pddABC-ddrAB</i> reverse
<i>chnA</i>	ChnA F	CATCAATTGACTACGTAG TCGTACGTGTAAGGAGGT TTGAAATGGAAAAATTAT	54	<i>chnA</i> forward

		G		
chnA	ChnA R	CATGCTAGCCCCGGGTAT CTTCTACTCATTTTTTATTT CG	55	chnA reverse
--	Top ter F1	CTAGAAGTCAAAAGCCTC CGACCGGAGGCTTTTGA	58	forward
--	Top ter F2	CTGCTCGAGTTGCTAGC AAGTTTAAACAAAAAAA GCCCGCTCATTAGGCGG GCTGAGCT	59	forward
--	Bot ter R1	CAGCCCGCCTAATGAGC GGGCTTTTTTTTGTAA AC	60	reverse
--	Bot ter R2	TTGCTAGCAACTCGAGCA GTCAAAGCCTCCGGTC GGAGGCTTTTGA	61	reverse
KA-AT	OT872	CTCCGGAATTCATGTCTG ACGGACGACTCACCGCA	127	Aminoalcohol kinase/lyase operon forward
KA-AT	OT873	TTCCAATGCATTGGCTGC AGTTATCTCTGTGCACGA GTGCCGATGA	128	Aminoalcohol kinase/lyase operon reverse
KA	OT879	AACAGCCAAGCTTGGCT GCAGTCATCGCGCATTCT CCGGG	129	Aminoalcohol kinase reverse
AT	OT880	TCTCCGGAATTCATGACG TCTGAAATGACAGCGACA GAAG	130	Aminoalcohol lyase forward
pBAD. HisB	OT909	GCTAACAGGAGGAAGAA TTCATGGGGGGTTCTC	131	Adds EcoRI site to replace NcoI site
pBAD. HisB	OT910	GAGAACCCCCCATGAATT CTTCCTCCTGTTAGC	132	Adds EcoRI site to replace NcoI site
BudAB	N84seqR3	GGACCTGCTTCGCTTTAT CG	159	reverse
APT	APTfor	GCGCGCCCGGGAAGAAG GAGCTCTTCACCATGAAC AAACCACAGTCTTGG	162	APT forward
APT	APTrev	GCGCGCCCGGGTTCATG CCACCTCTGCG	163	APT reverse

Table 5

Sequencing Primers

Name	Sequence	Gene-specific	SEQ ID NO:
M13 Forward	GTAAAACGACGGCCAGT	--	35
M13 Reverse	AACAGCTATGACCATG	--	36
N83 SeqF2	GCTGGATTACCAGCTCGACC	--	37
N83 SeqF3	CGGACGCATTACCGGCAAAG	--	38
N84 Seq R2	GCATCGAGATTATCGGGATG	--	65
N84 SeqR4	CGAAGCGAGAGAAGTTATCC	--	39

Trc F	TTGACAATTAATCATCCGGC	all	42
Trc R	CTTCTCTCATCCGCCAAAAC	all	43
DDko seq F2	GCATGGCGCGGATTTGACGAAC	pddABC- ddrAB	46
DDko seq F5	CATTAAAGAGACCAAGTACGTG	pddABC- ddrAB	47
DDko seq F7	ATATCCTGGTGGTGTCTCGTCGGCGT	pddABC- ddrAB	48
DDko seq F9	TCTTTGTCACCAACGCCCTGCG	pddABC- ddrAB	49
DDko seq R1	GCCCACCGCGCTCGCCGCCGCG	pddABC- ddrAB	50
DDko seq R3	CCCCCAGGATGGCGGCTTCGGC	pddABC- ddrAB	51
DDko seq R7	GGGCCGACGGCGATAATCACTT	pddABC- ddrAB	52
DDko seq R10	TTCTTCGATCCACTCCTTAACG	pddABC- ddrAB	53
chnSeq F1	CTCAACAGGGTGTAAGTGTAGT	chnA	56
chnSeq R1	CGTTTTGATATAGCCAGGATGT	chnA	57
pCL1925 vec F	CGGTATCATCAACAGGCTTACC	all	62
pCL1925 vec R1	AGGGTTTTCCAGTCACGACGT	all	63
pCL1925 vec R2	CGCAATAGTTGGCGAAGTAATC	all	64
APTseqRev	GCTAGAGATGATAGC	APT	160
APTseqFor	GGAAGAGACTATCCAGCG	APT	161

Methods for Determining 2-Butanol and 2-Butanone Concentration in Culture Media

The concentration of 2-butanol and 2-butanone in the culture media can be determined by a number of methods known in the art. For example, a specific high performance liquid chromatography (HPLC) method utilized a Shodex SH-1011 column with a Shodex SH-G guard column, both purchased from Waters Corporation (Milford, MA), with refractive index (RI) detection. Chromatographic separation was achieved using 0.01 M H₂SO₄ as the mobile phase with a flow rate of 0.5 mL/min and a column temperature of 50 °C. Under the conditions used, 2-butanone and 2-butanol had retention times of 39.5 and 44.3 min,

respectively. Alternatively, gas chromatography (GC) methods are available. For example, a specific GC method utilized an HP-INNOWax column (30 m x 0.53 mm id, 1 μ m film thickness, Agilent Technologies, Wilmington, DE), with a flame ionization detector (FID). The carrier gas was helium at a flow rate of 4.5 mL/min, measured at 150 °C with constant head pressure; injector split was 1:25 at 200 °C; oven temperature was 45 °C for 1 min, 45 to 220 °C at 10 °C/min, and 220 °C for 5 min; and FID detection was employed at 240 °C with 26 mL/min helium makeup gas. The retention times of 2-butanone and 2-butanol were 3.61 and 5.03 min, respectively.

2-Butanone can also be detected by derivatization with 3-methyl-2-benzothiazolinone hydrazone (MBTH). An aqueous solution containing 2-butanone is mixed with an equal volume of an aqueous solution of 6 mg/mL MBTH in 375 mM glycine-HCl (pH 2.7) and incubated at 100 °C for 3 min. The resulting MBTH-derivatized samples are analyzed on a 25 cm x 4.6 mm (id) Supelosil LC-18-D5 5 μ m column (Supelco) using a mobile phase of 55% acetonitrile in water at a flow rate of 1 mL/min. The 2-butanone derivative appears as two peaks (*cis* and *trans* isomers) with retention times of approximately 12.3 and 13.3 min and absorbance maxima of 230 and 307 nm.

The meaning of abbreviations is as follows: "s" means second(s), "min" means minute(s), "h" means hour(s), "psi" means pounds per square inch, "nm" means nanometers, "d" means day(s), " μ L" means microliter(s), "mL" means milliliter(s), "L" means liter(s), "mm" means millimeter(s), "nm" means nanometers, "mM" means millimolar, "M" means molar, "mmol" means millimole(s), " μ mol" means micromole(s)", "g" means gram(s), " μ g" means microgram(s) and "ng" means nanogram(s), "PCR" means polymerase chain reaction, "OD" means optical density, "OD₆₀₀" means the optical density measured at a wavelength of 600 nm, "kDa" means kilodaltons, "g" means the gravitation constant, "bp" means base pair(s), "kbp" means kilobase pair(s), "% w/v" means weight/volume percent, % v/v" means volume/volume percent, "wt %" means percent by weight, "HPLC" means high performance liquid chromatography, and "GC" means

gas chromatography. The term "molar selectivity" is the number of moles of product produced per mole of sugar substrate consumed and is reported as a percent.

EXAMPLE 1

5 Cloning and Expression of Acetolactate Synthase

The purpose of this Example was to clone and express in *E. coli* the *budB* gene that encodes the enzyme acetolactate synthase. The *budB* gene was amplified from *Klebsiella pneumoniae* strain ATCC 25955 genomic DNA using PCR.

10 The *budB* sequence which encodes acetolactate synthase was amplified from *Klebsiella pneumoniae* (ATCC 25955) genomic DNA by PCR using the primer pair B1 (SEQ ID NO:15) and B2 (SEQ ID NO:16). Other PCR amplification reagents (e.g. Kod HiFi DNA Polymerase (Novagen Inc., Madison, WI; catalog no. 71805-3)) were supplied in
15 manufacturers' kits and used according to the manufacturer's protocol. *Klebsiella pneumoniae* genomic DNA was prepared using the Gentra Puregene Puregene kit (Gentra Systems, Inc., Minneapolis, MN; catalog number D-5000A). Amplification was carried out in a DNA Thermocycler GeneAmp 9700 (PE Applied Biosystems, Foster city, CA). The nucleotide
20 sequence of the open reading frame (ORF) and the predicted amino acid sequence of the enzyme are given as SEQ ID NO:3 and SEQ ID NO:4, respectively.

For expression studies the Gateway cloning technology (Invitrogen Corp., Carlsbad, CA) was used. The entry vector pENTR/SD/D-TOPO
25 allows directional cloning and provided a Shine-Dalgarno sequence for the gene of interest. The destination vector pDEST14 used a T7 promoter for expression of the gene with no tag. The forward primer incorporated four bases (CACC) immediately adjacent to the translational start codon to allow directional cloning of the *budB* acetolactate synthase coding region
30 PCR product into pENTR/SD/D-TOPO (Invitrogen), generating the plasmid pENTRSDD-TOPObudB. The pENTR construct was transformed into *E. coli* Top10 (Invitrogen) cells and plated according to the manufacturer's recommendations. Transformants were grown overnight and plasmid

DNA was prepared using the QIAprep Spin Miniprep kit (Qiagen, Valencia, CA; catalog no. 27106) according to the manufacturer's recommendations. To create an expression clone, the budB coding region from pENTRSDD-TOPObudB was transferred to the pDEST 14 vector by *in vitro*

5 recombination using the LR Clonase mix (Invitrogen, Corp., Carlsbad, CA). The resulting vector, pDEST14budB, was transformed into BL-21-AI cells (Invitrogen Corp.). BL-21-AI cells carry a chromosomal copy of the T7 RNA polymerase under control of the arabinose-inducible araBAD promoter.

10 Transformants are inoculated into LB medium supplemented with 50 μ g/mL of ampicillin and grown overnight. An aliquot of the overnight culture is used to inoculate 50 mL of LB medium supplemented with 50 μ g/mL of ampicillin. The culture is incubated at 37 °C with shaking until the OD₆₀₀ reaches 0.6-0.8. The culture is split into two 25-mL portions and
15 arabinose is added to one of the flasks to a final concentration of 0.2% w/v. The negative control flask is not induced with arabinose. The flasks are incubated for 4 h at 37 °C with shaking. Cells are harvested by centrifugation and the cell pellets are resuspended in 50 mM MOPS, pH 7.0 buffer. The cells are disrupted either by sonication or by passage
20 through a French Pressure Cell. Each cell lysate is centrifuged yielding the supernatant and the pellet or the insoluble fraction. An aliquot of each fraction (whole cell lysate, from induced and control cells, is resuspended in SDS (MES) loading buffer (Invitrogen), heated to 85 °C for 10 min and subjected to SDS-PAGE analysis (NuPAGE 4-12% Bis-Tris Gel, catalog
25 no. NP0322Box, Invitrogen). A protein of the expected molecular weight, as deduced from the nucleic acid sequence, is present in the induced culture but not in the uninduced control.

Acetolactate synthase activity in the cell free extracts is measured using the method described by Bauerle et al. (Bauerle et al. (1964)
30 *Biochim. Biophys. Acta* 92:142-149). Protein concentration is measured by either the Bradford method or by the Bicinchoninic Kit (Sigma, catalog no. BCA-1; St. Louis, MO) using Bovine serum albumin (BSA) (Bio-Rad, Hercules, CA) as the standard.

EXAMPLE 2

Cloning and Expression of Acetolactate Decarboxylase

The purpose of this Example was to clone and express in *E. coli* the *budA* gene that encodes the enzyme acetolactate decarboxylase. The
5 *budA* gene was amplified from *Klebsiella pneumoniae* strain ATCC 25955 genomic DNA using PCR.

The *budA* sequence which encodes acetolactate decarboxylase, was cloned in the same manner as described for *budB* in Example 1, except that the primers used for PCR amplification were B3 (SEQ ID
10 NO:17) and B4 (SEQ ID NO:18). The nucleotide sequence of the open reading frame (ORF) and the predicted amino acid sequence of the enzyme are given as SEQ ID NO:1 and SEQ ID NO:2, respectively. The resulting plasmid was named pENTRSDD-TOPO*budA*.

Acetolactate decarboxylase activity in the cell free extracts is
15 measured using the method described by Bauerle et al., *supra*.

EXAMPLE 3 (Prophetic)

Cloning and Expression of Butanediol Dehydrogenase

The purpose of this prophetic Example is to describe how to clone and express in *E. coli* the *budC* gene that encodes the enzyme butanediol
20 dehydrogenase. The *budC* gene is amplified from *Klebsiella pneumoniae* strain IAM1063 genomic DNA using PCR.

The *budC* sequence encoding butanediol dehydrogenase is cloned and expressed in the same manner as described for *budA* in Example 1, except that the primers used for PCR amplification are B5 (SEQ ID NO:19)
25 and B6 (SEQ ID NO:20) and the genomic template DNA is from *Klebsiella pneumoniae* IAM1063 (which is obtained from the Institute of Applied Microbiology Culture Collection, Tokyo, Japan). *Klebsiella pneumoniae* IAM1063 genomic DNA is prepared using the Gentra Puregene Puregene kit (Gentra Systems, Inc., Minneapolis, MN; catalog number D-5000A).
30 The nucleotide sequence of the open reading frame (ORF) and the predicted amino acid sequence of the enzyme are given as SEQ ID NO:5 and SEQ ID NO:6, respectively.

Butanediol dehydrogenase activity in the cell free extracts is measured spectrophotometrically by following NADH consumption at an absorbance of 340 nm.

EXAMPLE 4 (Prophetic)

5 Cloning and Expression of Butanediol Dehydratase

The purpose of this prophetic Example is to describe how to clone and express in *E. coli* the *pddA*, *pddB* and *pddC* genes that encode butanediol dehydratase. The *pddA*, *pddB* and *pddC* genes are amplified from *Klebsiella oxytoca* ATCC 8724 genomic DNA using PCR.

10 The *pddA*, *pddB* and *pddC* sequences which encode butanediol dehydratase are cloned and expressed in the same manner as described for *budA* in Example 1, except that the genomic template DNA is from *Klebsiella oxytoca* ATCC 8724, and the primers are B7 (SEQ ID NO:21) and B8 (SEQ ID NO:22). *Klebsiella oxytoca* genomic DNA is prepared
15 using the Gentra Puregene Puregene kit (Gentra Systems, Inc., Minneapolis, MN; catalog number D-5000A). A single PCR product including all three open reading frames (ORFs) is cloned, so that all three coding regions are expressed as an operon from a single promoter on the expression plasmid. The nucleotide sequences of the open reading frames
20 for the three subunits are given as SEQ ID NOs:7, 9, and 11, respectively, and the predicted amino acid sequences of the three enzyme subunits are given as SEQ ID NOs:8, 10, and 12, respectively.

Butanediol dehydratase activity in the cell free extracts is measured by derivatizing the ketone product with 2,4-dinitrophenylhydrazine
25 (DNPH). Briefly, 100 μ L of reaction mixture, cell extract containing approximately 0.0005 units of enzyme, 40 mM potassium phosphate buffer (pH 8.0), 2 μ g of adenosylcobalamin, 5 μ g of 2,3,-butanediol, and 1 μ g of bovine serum albumin, is quenched by addition of an equal volume of 0.05 wt % DNPH in 1.0 N HCl. After 15 min at room temperature, the
30 color is developed by addition of 100 μ L of 4 N NaOH. The amount of product is determined from the absorbance of the final solution at 550 nm compared to a standard curve prepared with 2-butanone. All reactions are carried out at 37 °C under dim red light.

EXAMPLE 5 (Prophetic)

Cloning and Expression of Butanol Dehydrogenase

The purpose of this prophetic Example is to describe how to clone and express in *E. coli* the *sadh* gene that encodes butanol dehydrogenase. The *sadh* gene is amplified from *Rhodococcus ruber* strain 219 genomic DNA using PCR.

The *sadh* sequence encoding butanol dehydrogenase is cloned and expressed in the same manner as described for *budA* in Example 1, except that the genomic template DNA is from *Rhodococcus ruber* strain 219 (Meens, Institut fuer Mikrobiologie, Universitaet Hannover, Hannover, Germany) and the primers are B9 (SEQ ID NO:23) and B10 (SEQ ID NO:24). *Rhodococcus ruber* genomic DNA is prepared using the Ultra Clean™ Microbial DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA), according to the manufacturer's protocol. The nucleotide sequence of the open reading frame (ORF) and the predicted amino acid sequence of the enzyme are given as SEQ ID NO:13 and SEQ ID NO:14, respectively.

Butanol dehydrogenase activity in cell free extracts is measured by following the increase in absorbance at 340 nm resulting from the conversion of NAD to NADH when the enzyme is incubated with NAD and 2-butanol.

EXAMPLE 6 (Prophetic)

Construction of a Transformation Vector for the Genes in a 2-Butanol Biosynthetic Pathway

The purpose of this prophetic Example is to describe the preparation of a transformation vector for the genes in a 2-butanol biosynthetic pathway (i.e., Pathway 3 as described above). Like most organisms, *E. coli* converts glucose initially to pyruvic acid. The enzymes required to convert pyruvic acid to 2-butanol following Pathway 3, i.e., acetolactate synthase, acetolactate decarboxylase, butanediol dehydrogenase, butanediol dehydratase, and butanol dehydrogenase, are encoded by the *budA*, *budB*, *budC*, *pddA*, *pddB*, *pddC* and *sadh* genes. To simplify building the 2-butanol biosynthetic pathway in a recombinant

organism, the genes encoding the 5 steps in the pathway are divided into two operons. The upper pathway comprises the first three steps catalyzed by acetolactate synthase, acetolactate decarboxylase, and butanediol dehydrogenase. The lower pathway comprises the last two steps catalyzed by butanediol dehydratase and butanol dehydrogenase.

The coding sequences are amplified by PCR with primers that incorporate restriction sites for later cloning, and the forward primers contain an optimized *E. coli* ribosome binding site (AAAGGAGG). PCR products are TOPO cloned into the pCR4Blunt-TOPO vector and transformed into Top10 cells (Invitrogen). Plasmid DNA is prepared from the TOPO clones, and the sequence of the cloned PCR fragment is verified. Restriction enzymes and T4 DNA ligase (New England Biolabs, Beverly, MA) are used according to manufacturer's recommendations. For cloning experiments, restriction fragments are gel-purified using QIAquick Gel Extraction kit (Qiagen).

After confirmation of the sequence, the coding regions are subcloned into a modified pUC19 vector as a cloning platform. The pUC19 vector is modified by a HindIII/SapI digest, followed by treatment with Klenow DNA polymerase to fill in the ends. The 2.4 kB vector fragment is gel-purified and religated creating pUC19dHS. Alternatively the pUC19 vector is modified by a SphI/SapI digest, followed by treatment with Klenow DNA polymerase to blunt the ends. The 2.4 kB vector fragment is gel-purified and religated creating pUC19dSS. The digests remove the lac promoter adjacent to the MCS (multiple cloning sites), preventing transcription of the operons from the vector.

Upper Pathway:

The *budABC* coding regions are amplified from *Klebsiella pneumoniae* genomic DNA by PCR using primer pair B11 and B12 (Table 4), given as SEQ ID NOs:25 and 26, respectively. The forward primer incorporates an EcoRI restriction site and a ribosome binding site (RBS). The reverse primer incorporates an SphI restriction site. The PCR product is cloned into pCR4 Blunt-TOPO creating pCR4 Blunt-TOPO-budABC.

To construct the upper pathway operon pCR4 Blunt-TOPO-budABC is digested with EcoRI and SphI releasing a 3.2 kbp *budABC* fragment. The pUC19dSS vector is also digested with EcoRI and SphI, releasing a 2.0 kbp vector fragment. The *budABC* fragment and the vector fragment
 5 are ligated together using T4 DNA ligase (New England Biolabs) to form pUC19dSS-budABC.

Lower Pathway:

The *pddABC* coding regions are amplified from *Klebsiella oxytoca* ATCC 8724 genomic DNA by PCR using primers B13 and B14 (Table 4),
 10 given as SEQ ID NOs:27 and 28, respectively, creating a 2.9 kbp product. The forward primer incorporates EcoRI and PmeI restriction sites and a RBS. The reverse primer incorporates the BamHI restriction site. The PCR product is cloned into pCRBlunt II-TOPO creating pCRBluntII-pdd.

The *sadh* gene is amplified from *Rhodococcus ruber* strain 219 genomic DNA by PCR using primers B15 and B16 (Table 4), given as
 15 SEQ ID NOs:29 and 30, respectively, creating a 1.0 kbp product. The forward primer incorporates a BamHI restriction site and a RBS. The reverse primer incorporates an XbaI restriction site. The PCR product is cloned into pCRBlunt II-TOPO creating pCRBluntII-sadh.

20 To construct the lower pathway operon, a 2.9 kbp EcoRI and BamHI fragment from pCRBluntII-pdd, a 1.0 kbp BamHI and XbaI fragment from pCRBluntII-sadh, and the large fragment from an EcoRI and XbaI digest of pUC19dHS are ligated together. The three-way ligation creates pUC19dHS-pdd-sadh.

25 The pUC19dSS-budABC vector is digested with PmeI and HindIII, releasing a 3.2 kbp fragment that is cloned into pBenBP, an *E.coli-B. subtilis* shuttle vector. Plasmid pBenBP is created by modification of the pBE93 vector, which is described by Nagarajan (WO 93/2463, Example 4). To generate pBenBP, the *Bacillus amyloliquefaciens* neutral protease promoter (NPR) signal sequence and the *phoA* gene are removed from
 30 pBE93 with an NcoI/HindIII digest. The NPR promoter is PCR amplified from pBE93 by primers BenF and BenBPR, given by SEQ ID NOs:31 and 32, respectively. Primer BenBPR incorporates BstEII, PmeI and HindIII

sites downstream of the promoter. The PCR product is digested with NcoI and HindIII, and the fragment is cloned into the corresponding sites in the vector pBE93 to create pBenBP. The upper operon fragment is subcloned into the PmeI and HindIII sites in pBenBP creating pBen-budABC.

- 5 The pUC19dHS-pdd-sadh vector is digested with PmeI and HindIII releasing a 3.9 kbp fragment that is cloned into the PmeI and HindIII sites of pBenBP, creating pBen-pdd-sadh.

EXAMPLE 7 (Prophetic)

Expression of a 2-Butanol Biosynthetic Pathway in *E. coli*

- 10 The purpose of this prophetic Example is to describe how to express a 2-butanol biosynthetic pathway in *E. coli*.

The plasmids pBen-budABC and pBen-pdd-sadh, prepared as described in Example 6, are separately transformed into *E. coli* NM522 (ATCC No. 47000), and expression of the genes in each operon is
 15 monitored by SDS-PAGE analysis and enzyme assay. After confirmation of expression of all genes, pBen-budABC is digested with EcoRI and HindIII to release the NPR promoter-budABC fragment. The fragment is blunt ended using the Klenow fragment of DNA polymerase (New England Biolabs, catalog no. M0210S). The plasmid pBen-pdd-sadh is digested
 20 with EcoRI and similarly blunted to create a linearized, blunt-ended vector fragment. The vector and NPR-budABC fragments are ligated, creating p2BOH. This plasmid is transformed into *E. coli* NM522 to give *E. coli* NM522/p2BOH, and expression of the genes is monitored as previously described.

- 25 *E. coli* NM522/p2BOH is inoculated into a 250 mL shake flask containing 50 mL of medium and shaken at 250 rpm and 35 °C. The medium is composed of: dextrose, 5 g/L; MOPS, 0.05 M; ammonium sulfate, 0.01 M; potassium phosphate, monobasic, 0.005 M; S10 metal mix, 1% (v/v); yeast extract, 0.1% (w/v); casamino acids, 0.1% (w/v);
 30 thiamine, 0.1 mg/L; proline, 0.05 mg/L; and biotin 0.002 mg/L, and is titrated to pH 7.0 with KOH. S10 metal mix contains: MgCl₂, 200 mM; CaCl₂, 70 mM; MnCl₂, 5 mM; FeCl₃, 0.1 mM; ZnCl₂, 0.1 mM; thiamine hydrochloride, 0.2 mM; CuSO₄, 172 μM; CoCl₂, 253 μM; and Na₂MoO₄,

242 μ M. After 18 h, 2-butanol is detected by HPLC or GC analysis using methods that are well known in the art, for example, as described in the General Methods section above.

EXAMPLE 8 (Prophetic)

5 Expression of a 2-Butanol Biosynthetic Pathway in *Bacillus subtilis*

The purpose of this prophetic Example is to describe how to express a 2-butanol biosynthetic pathway in *Bacillus subtilis*.

The plasmids pBen-budABC and pBen-pdd-sadh, prepared as described in Example 6, are separately transformed into *Bacillus subtilis*
10 BE1010 (*J. Bacteriol.* 173:2278-2282 (1991)) and expression of the genes in each operon is monitored as described in Example 7. The plasmid pBen-budABC is digested with EcoRI and HindIII to release the NPR promoter-budABC fragment. The fragment is blunt ended using the Klenow fragment of DNA polymerase (New England Biolabs, catalog no.
15 M0210S). The plasmid pBen-pdd-sadh is digested with EcoRI and similarly blunted to create a linearized, blunt-ended vector fragment. The vector and NPR-budABC fragments are ligated, creating p2BOH. This plasmid is transformed into *Bacillus subtilis* BE1010 to give *Bacillus subtilis* BE1010 /p2BOH, and expression of the genes is monitored as
20 previously described.

Bacillus subtilis BE1010 /p2BOH is inoculated into a 250 mL shake flask containing 50 mL of medium and shaken at 250 rpm and 35 °C for 18 h. The medium is composed of: dextrose, 5 g/L; MOPS, 0.05 M; glutamic acid, 0.02 M; ammonium sulfate, 0.01 M; potassium phosphate,
25 monobasic buffer, 0.005 M; S10 metal mix (as described in Example 7), 1% (v/v); yeast extract, 0.1% (w/v); casamino acids, 0.1% (w/v); tryptophan, 50 mg/L; methionine, 50 mg/L; and lysine, 50 mg/L, and is titrated to pH 7.0 with KOH. After 18 h, 2-butanol is detected by HPLC or GC analysis using methods that are well known in the art, for example, as
30 described in the General Methods section above.

EXAMPLE 9

Construction of a Transformation Vector for the Genes in a 2-Butanol Biosynthetic Pathway

The purpose of this Example was to prepare a recombinant *E. coli* host carrying the genes in a 2-butanol biosynthetic pathway (i.e., Pathway 3 as described above). Like most organisms, *E. coli* converts glucose initially to pyruvic acid. The enzymes required to convert pyruvic acid to 2-butanone in Pathway 3, i.e., acetolactate synthase, acetolactate decarboxylase, butanediol dehydrogenase, and butanediol dehydratase are encoded by the *budA*, *budB*, *budC*, *pddA*, *pddB*, and *pddC* genes. In the last step of the pathway, a butanol dehydrogenase converts 2-butanone to 2-butanol. Dehydrogenases that carry out this last step are promiscuous and may be found in many organisms. To simplify building the 2-butanol biosynthetic pathway in a recombinant organism, the genes encoding the 5 steps in the pathway were divided into multiple operons. The upper pathway operon comprised the first three steps catalyzed by acetolactate synthase, acetolactate decarboxylase, and butanediol dehydrogenase and were cloned onto an expression vector. The lower pathway comprised the last two steps catalyzed by butanediol dehydratase including the reactivating factor (Mori et al., *J. Biol. Chem.* 272:32034 (1997)) and a butanol dehydrogenase. The diol dehydratase can undergo suicide inactivation during catalysis. The reactivating factor protein encoded by *ddrA* and *ddrB* (GenBank AF017781, SEQ ID NO:70) reactivates the inactive enzyme. The *ddrA* and *ddrB* genes flank the diol dehydratase operon. The operons for the dehydratase/reactivating factor and the butanol dehydrogenase were either cloned onto another expression vector or the dehydratase/reactivating factor operon was cloned singly onto another expression vector and the last step was provided by an endogenous activity in the demonstration host.

Construction of Vector pTrc99a-budABC:

The *budAB* coding regions were amplified from *K. pneumoniae* ATCC 25955 genomic DNA by PCR using primer pair BABC F and BAB R, given as SEQ ID NOs:33 and 34, respectively (see Table 4), creating a

2.5 kbp product. The forward primer incorporated SacI and EcoRI restriction sites and a ribosome binding site (RBS). The reverse primer incorporated a SpeI restriction site. The PCR product was cloned into pCR4 Blunt-TOPO creating pCR4 Blunt-TOPO-budAB. Plasmid DNA was prepared from the TOPO clones and the sequence of the genes was verified with primers M13 Forward (SEQ ID NO:35), M13 Reverse (SEQ ID NO:36), N83 SeqF2 (SEQ ID NO:37), N83 SeqF3 (SEQ ID NO:38) and N84 SeqR4 (SEQ ID NO:39) (see Table 5).

The *budC* coding region was amplified from *K. pneumoniae* ATCC 25955 genomic DNA by PCR using primer pair BC Spe F and BC Xba R given as SEQ ID NOs:40 and 41, respectively, creating a 0.8 kbp product. The forward primer incorporated a SpeI restriction site, a RBS and modified the CDS by changing the second and third codons from AAA to AAG. The reverse primer incorporated an XbaI restriction site. The PCR product was cloned into pCR4 Blunt-TOPO creating pCR4 Blunt-TOPO-budC. Plasmid DNA was prepared from the TOPO clones and the sequence of the genes was verified with primers M13 Forward (SEQ ID NO:35) and M13 Reverse (SEQ ID NO:36).

To construct the budABC operon, pCR4 Blunt-TOPO-budC was digested with SnaBI and XbaI releasing a 1.0 kbp *budC* fragment. The vector pTrc99a (Amann et al., *Gene* 69(2):301-315 (1988)) was digested with SmaI and XbaI creating a 4.2 kbp linearized vector fragment. The vector and the budC fragment were ligated to create pTrc99a-budC and transformed into *E. coli* Top 10 cells (Invitrogen). Transformants were analyzed by PCR amplification with primers Trc F (SEQ ID NO:42) and Trc R (SEQ ID NO:43) for a 1.2 kbp product to confirm the presence of the *budC* insert. The *budAB* genes were subcloned from pCR4 Blunt-TOPO-budAB as a 2.5 kbp EcoRI/SpeI fragment. Vector pTrc99a-budC was digested with EcoRI and SpeI and the resulting 5.0 kbp vector fragment was gel-purified. The purified vector and budAB insert were ligated and transformed into *E. coli* Top 10 cells. Transformants were screened by PCR amplification with primers Trc F (SEQ ID NO:42) and N84 Seq R2 (SEQ ID NO:65) to confirm creation of pTrc99a-budABC. In this plasmid,

the bud A, B, and C coding regions are adjacent to each other, in this order, and between the Trc promoter and the *rrnB* termination sequence.

Results:

Three independent isolates of *E. coli* Top 10/pTrc99a-budABC were examined for the production of butanediol, using *E. coli* Top 10/pCL1925-Kodd-ddr (described below) as a negative control. The strains were grown in LB medium containing 100 µg/mL carbenicillin. The resulting cells were used to inoculate shake flasks (approximately 175 mL total volume) containing 125 mL of TM3a/glucose medium with 100 µg/mL carbenicillin. In addition, the flasks inoculated with strains carrying pTrc99a-budABC contained 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). TM3a/glucose medium contains (per liter): 10 g glucose, 13.6 g KH₂PO₄, 2.0 g citric acid monohydrate, 3.0 g (NH₄)₂SO₄, 2.0 g MgSO₄·7H₂O, 0.2 g CaCl₂·2H₂O, 0.33 g ferric ammonium citrate, 1.0 mg thiamine·HCl, 0.50 g yeast extract, and 10 mL trace elements solution, adjusted to pH 6.8 with NH₄OH. The solution of trace elements contained: citric acid·H₂O (4.0 g/L), MnSO₄·H₂O (3.0 g/L), NaCl (1.0 g/L), FeSO₄·7H₂O (0.10 g/L), CoCl₂·6H₂O (0.10 g/L), ZnSO₄·7H₂O (0.10 g/L), CuSO₄·5H₂O (0.010 g/L), H₃BO₃ (0.010 g/L), and Na₂MoO₄·2H₂O (0.010 g/L). The flasks, capped with vented caps, were inoculated at a starting OD₆₀₀ of approximately 0.03 units and incubated at 34 °C with shaking at 300 rpm.

Approximately 23 h after induction, an aliquot of the broth was analyzed by HPLC (Shodex Sugar SH1011 column) and GC (HP-INNOWax), using the same methods described in the General Methods section for 2-butanol and 2-butanone. The results of the analysis are given in Table 6. The three *E. coli* clones converted glucose to acetoin and *meso*-2,3-butanediol, the desired intermediates of the pathway, with a molar selectivity of 14%. This selectivity was approximately 35-fold higher than that observed with the *E. coli* control strain lacking budABC.

Table 6
Production of Acetoin and meso-2,3-butanediol
by *E. coli* Top 10/pTrc99a-budABC

Strain	OD ₆₀₀	Acetoin, mM	Meso-2,3-Butanediol, mM	Molar Selectivity ^a , %
Negative control	1.4	0.07	0.03	0.4
Isolate #1	1.5	0.64	1.3	14
Isolate #2	1.4	0.70	1.2	14
Isolate #3	1.4	0.74	1.3	15

5 ^a Molar selectivity is (acetoin + meso-2,3-butanediol)/(glucose consumed).

Construction of vector pCL1925-KoDD-ddr:

The diol dehydratase (GenBank D45071, SEQ ID NO:69) and reactivating factor (GenBank AF017781, SEQ ID NO:70) operons were
10 PCR amplified from *Klebsiella oxytoca* ATCC 8724 as a single unit with primers DDo For (SEQ ID NO: 44) and DDo Rev (SEQ ID NO:45). The forward primer incorporated an optimized *E. coli* RBS and a HindIII restriction site. The reverse primer included an XbaI restriction site. The 5318 bp PCR product was cloned into pCR4Blunt-TOPO and clones of the
15 resulting pCR4Blunt-TOPO-Kodd-ddr were sequenced with primers M13 Forward (SEQ ID NO:35), M13 Reverse (SEQ ID NO:36), DDko seq F2 (SEQ ID NO:46), DDko seq F5 (SEQ ID NO:47), DDko seq F7 (SEQ ID NO:48), DDko seq F9 (SEQ ID NO:49), DDko seq R1 (SEQ ID NO:50), DDko seq R3 (SEQ ID NO:51), DDko seq R7 (SEQ ID NO:52), and DDko
20 seq R10 (SEQ ID NO:53). A clone having the insert with the expected sequence was identified.

For expression, the diol dehydratase/reactivating factor genes were subcloned into pCL1925 (U.S. Patent No. 7,074,608), a low copy plasmid carrying the glucose isomerase promoter from *Streptomyces*. pCR4Blunt-TOPO-Kodd-ddr was digested with HindIII and XbaI and the resulting 5.3 kbp Kodd-ddr fragment was gel-purified. Vector pCL1925 was digested with HindIII and XbaI and the resulting 4539 bp vector fragment was gel purified. The vector and Kodd-ddr fragment were ligated and transformed into *E. coli* Top10. Transformants were screened by PCR with primers DDko Seq F7 (SEQ ID NO:48) and DDko Seq R7 (SEQ ID NO: 52). Amplification of the plasmid (pCL1925-Kodd-ddr) carrying the insert resulted in a product of approximately 797 bp.

Activity of diol dehydratase towards *meso*-2,3-butanediol was measured by incubating cell extract (total protein ~ 0.8 mg/mL) with 10 mM butanediol and 12 mM coenzyme B₁₂ in 80 mM HEPES (pH 8.2) for 17 h at room temperature. Formation of the expected product, 2-butanone, was determined by HPLC as described in the General Methods.

Construction of vector pCL1925-KoDD-ddr::T5 chnA ter:

To provide a heterologous alcohol dehydrogenase activity, the *chnA* gene encoding cyclohexanol dehydrogenase from *Acinetobacter* sp. (Cheng et al., *J. Bacteriol.* 182:4744-4751 (2000)) was cloned into the pCL1925 vector with the diol dehydratase operon, pCL1925-Kodd-ddr. The *chnA* gene, given as SEQ ID NO:71 (Genbank No: AF282240, SEQ ID NO:73) was amplified from pDCQ2, a cosmid carrying the cyclohexanol gene cluster from *Acinetobacter*, with primers ChnA F (SEQ ID NO:54) and ChnA R (SEQ ID NO:55). The resulting 828 bp PCR product was cloned into pCR4Blunt-TOPO to create pCR4Blunt-TOPO-chnA and transformants were screened by colony PCR with primers M13 Forward (SEQ ID NO:35) and M13 Reverse (SEQ ID NO:36). Correct clones produced a PCR product of about 1 kbp and were sequenced with primers M13 Forward (SEQ ID NO:35) and M13 Reverse (SEQ ID NO:36).

After sequencing pCR4Blunt-TOPO-chnA to confirm the correct sequence, the *chnA* gene was subcloned from the plasmid as an 813 bp MfeI/SmaI fragment. The expression vector pQE30 (Qiagen) was digested

with MfeI and SmaI and the resulting 3350 bp vector fragment was gel-purified. The *chnA* fragment and the purified vector were ligated and transformed into *E. coli* Top10 cells. Transformants were colony PCR screened with primers chnSeq F1 (SEQ ID NO:56) and chnseq R1 (SEQ ID NO:57) for a 494 bp PCR product. This cloning placed the *chnA* gene under the control of the T5 promoter in the plasmid, pQE30-*chnA*.

To prepare the pCL1925 vector to carry two operons, terminators were added to the vector. A tonB terminator-mcs-trpA terminator fragment was prepared by oligonucleotide annealing with primers Top ter F1 (SEQ ID NO:58), Top ter F2 (SEQ ID NO:59), Bot ter R1 (SEQ ID NO:60) and Bot ter R2 (SEQ ID NO:61). The annealed DNA was gel-purified on a 6% PAGE gel (Embi-tec, San Diego, CA). Vector pCL1925 was digested with SacI and XbaI and gel-purified. The annealed DNA and vector fragment were ligated to create pCL1925-ter. Transformants were screened by colony PCR amplification with primers pCL1925 vec F (SEQ ID NO:62) and pCL1925 vec R1 (SEQ ID NO:63) for the presence of a PCR product of approximately 400 bp. Positive clones from the PCR screen were sequenced with the same primers.

Vector pCL1925-ter was digested with XhoI and PmeI and the resulting 4622 bp fragment was gel-purified. pQE30-*chnA* was digested with NcoI and the DNA was treated with Klenow DNA polymerase to blunt the ends. pQE30-*chnA* was then digested with XhoI and the resulting 1.2 kbp T5 promoter-*chnA* fragment was gel-purified. The pCL1925-ter vector and the *chnA* operon fragment were ligated together to give pCL1925-ter-T5*chnA* and transformed into *E. coli* Top10. Transformants were screened by colony PCR amplification with primers pCL1925 vec F (SEQ ID NO:64) and chnseq R1 (SEQ ID NO:59) for a product of approximately 1 kbp.

To finish building the pathway vector, the pCL1925-KoDD-ddr plasmid was digested with XbaI and SacI and the resulting 9504 bp vector fragment was gel-purified. The *chnA* operon flanked by terminators, with the trpA terminator (Koichi et al. (1997) Volume 272, Number 51, pp. 32034-32041) 3' to the *chnA* coding sequence, from pCL1925-ter-T5*chnA*

was gel-purified as a 1271 bp XbaI/SacI fragment. After ligation of the fragments and transformation into *E. coli* Top10, transformants were screened by colony PCR. Primers chnSeq F1 (SEQ ID NO:58) and pCL1925 vec R2 (SEQ ID NO:64) amplified the expected 1107 bp PCR
5 product in the resulting plasmid, pCL1925-KoDD-ddr::ter-T5chnA .

EXAMPLE 10

Expression of a 2-Butanol Biosynthetic Pathway in *E. coli* with Overexpressed Endogenous Alcohol Dehydrogenase

The purpose of this Example was to express a 2-butanol
10 biosynthetic pathway in several *E. coli* strains.

Construction of *E. coli* strains constitutively expressing *yqhD*:

E. coli contains a native gene (*yqhD*) that was identified as a 1,3-propanediol dehydrogenase (U.S. Patent No. 6,514,733). The *yqhD* gene, given as SEQ ID NO:74, has 40% identity to the gene *adhB* in *Clostridium*,
15 a probable NADH-dependent butanol dehydrogenase. The *yqhD* gene was placed under the constitutive expression of a variant of the glucose isomerase promoter 1.6GI (SEQ ID NO:67) in *E. coli* strain MG1655 1.6yqhD::Cm (WO 2004/033646) using λ Red technology (Datsenko and Wanner, *Proc. Natl. Acad. Sci. U.S.A.* 97:6640 (2000)). Similarly, the
20 native promoter was replaced by the 1.5GI promoter (WO 2003/089621) (SEQ ID NO:68), creating strain MG1655 1.5yqhD::Cm, thus, replacing the 1.6GI promoter of MG1655 1.6yqhD::Cm with the 1.5GI promoter. The 1.5GI and 1.6GI promoters differ by 1 bp in the -35 region, thereby altering the strength of the promoters (WO 2004/033646). While replacing the
25 native *yqhD* promoter with either the 1.5GI or 1.6GI promoter, the *yqhC* gene encoding the putative transcriptional regulator for the *yqh* operon was deleted. Butanol dehydrogenase activity was confirmed by enzyme assay using methods that are well known in the art.

Transformation of *E. coli* strains:

30 Pathway plasmids pCL1925-Kodd-ddr and pTrc99a-budABC, described in Example 9, were co-transformed into *E. coli* strains MG1655, MG1655 1.6yqhD, and MG1655 1.5yqhD. The two latter strains overexpress the 1,3-propanediol dehydrogenase, YqhD, which also has

butanol dehydrogenase activity. Strains were examined for the production of 2-butanone and 2-butanol essentially as described above. Cells were inoculated into shake flasks (approximately 175 mL total volume) containing either 50 or 150 mL of TM3a/glucose medium (with 0.1 mg/L vitamin B₁₂, appropriate antibiotics and IPTG) to represent medium and low oxygen conditions, respectively. Spectinomycin (50 µg/mL) and carbenicillin (100 µg/mL) were used for plasmids pCL1925-Kodd-ddr and pTrc99a-budABC, respectively. The flasks were inoculated at a starting OD₆₀₀ of ≤ 0.04 units and incubated at 34 °C with shaking at 300 rpm. The flasks containing 50 mL of medium were capped with vented caps; the flasks containing 150 mL, were capped with non-vented caps to minimize air exchange. IPTG was present at time zero at a concentration of zero or 0.04 mM. Analytical results for 2-butanone and 2-butanol production are presented in Table 7. All the *E. coli* strains comprising a 2-butanol biosynthetic pathway produced 2-butanone under low and medium oxygen conditions and produced 2-butanol under low oxygen conditions.

Table 7

Production of 2-Butanone and 2-Butanol by *E. coli* MG1655 strains harboring pathway plasmids pCL1925-Kodd-ddr and pTrc99a-budABC

<u>Strain^{a,b}</u>	<u>IPTG, mM</u>	<u>Volume of Medium, mL</u>	<u>2-Butanone, mM</u>	<u>2-Butanol, mM</u>
MG1655 #1	0	50	0.08	Not detected
MG1655 #2	0	50	0.11	Not detected
MG1655 #1	0.04	50	0.12	Not detected
MG1655 #2	0.04	50	0.11	Not detected
MG1655 #1	0	150	0.15	0.047
MG1655 #2	0	150	0.19	0.041
MG1655 #1	0.04	150	0.10	0.015
MG1655 #2	0.04	150	0.11	0.015
MG1655 1.5yqhD #1	0	50	0.10	Not detected

MG1655 1.5yqhD #2	0	50	0.07	Not detected
MG1655 1.5yqhD #1	0.04	50	0.12	Not detected
MG1655 1.5yqhD #2	0.04	50	0.18	Not detected
MG1655 1.5yqhD #1	0	150	0.16	0.030
MG1655 1.5yqhD #2	0	150	0.18	0.038
MG1655 1.5yqhD #1	0.04	150	0.10	0.021
MG1655 1.5yqhD #2	0.04	150	0.09	0.017
MG1655 1.6yqhD #1	0	50	0.08	Not detected
MG1655 1.6yqhD #2	0	50	0.07	Not detected
MG1655 1.6yqhD #1	0.04	50	0.12	Not detected
MG1655 1.6yqhD #2	0.04	50	0.15	Not detected
MG1655 1.6yqhD #1	0	150	0.17	0.019
MG1655 1.6yqhD #2	0	150	0.18	0.041
MG1655 1.6yqhD #1	0.04	150	0.11	0.026
MG1655 1.6yqhD #2	0.04	150	0.11	0.038
Control (uninoculated medium)			Not detected	Not detected

^a #1 and #2 represent independent isolates.

^b MG1655 is MG1655/pCL1925-Kodd-ddr/pTrc99a-budABC
 MG1655 1.6yqhD is MG1655 1.6yqhD/pCL1925-Kodd-ddr/pTrc99a- budABC
 MG1655 1.6yqhD is MG1655 1.5yqhD/pCL1925-Kodd-ddr/pTrc99a- budABC.

5

EXAMPLE 11

Expression of a 2-Butanol Biosynthetic Pathway in *E. coli* with
 Heterologous Alcohol Dehydrogenase

Plasmids pCL1925-KoDD-ddr::ter-T5chnA and pTrc99a-budABC,
 described in Example 9, were transformed into *E. coli* strains MG1655 and
 10 MG1655 Δ yqhCD for a demonstration of the production of 2-butanol.

MG1655 Δ yqhCD carries a *yqhCD* inactivation that was made using
 the method of Datsenko and Wanner (*Proc. Natl. Acad. Sci. U.S.A.*
 97(12):6640-6645 (2000)). After replacement of the region with the FRT-
 CmR-FRT cassette of pKD3, the chloramphenicol resistance marker was
 15 removed using the FLP recombinase. The sequence of the deleted region
 is given as SEQ ID NO:66.

Strains MG1655/pTrc99a-budABC/pCL1925KoDD-ddr::ter-T5 chnA
 and MG1655 Δ yqhCD/pTrc99a-budABC/pCL1925KoDD-ddr::ter-T5 chnA
 were examined for the production of 2-butanone and 2-butanol essentially
 20 as described above. Strain MG1655 Δ yqhCD/pCL1925 was used as a
 negative control. Cells were inoculated into shake flasks (approximately
 175 mL total volume) containing 50 or 150 mL of TM3a/glucose medium
 (with 0.1 mg/L vitamin B₁₂ and appropriate antibiotics) to represent
 medium and low oxygen conditions, respectively. Spectinomycin (50
 25 μ g/mL) and ampicillin (100 μ g/mL) were used for selection of pCL1925
 based plasmids and pTrc99a-budABC, respectively. Enzyme activity
 derived from pTrc99a-budABC was detected by enzyme assay in the
 absence of IPTG inducer, thus, IPTG was not added to the medium. The
 flasks were inoculated at a starting OD₆₀₀ of ≤ 0.01 units and incubated at
 30 34 °C with shaking at 300 rpm for 24 h. The flasks containing 50 mL of
 medium were capped with vented caps; the flasks containing 150 mL,
 were capped with non-vented caps to minimize air exchange. Analytical
 results for 2-butanone and 2-butanol production are presented in Table 8.
 Both *E. coli* strains comprising a 2-butanol biosynthetic pathway produced

2-butanone under low and medium oxygen conditions and produced 2-butanol under low oxygen conditions, while the negative control strain did not produce detectable levels of either 2-butanone or 2-butanol.

Table 8

5

Production of 2-butanone and 2-butanol by *E. coli* strains

Strain ^a	Volume, mL	2-Butanone, mM	2-Butanol, mM
Negative control, MG1655 $\Delta yqhCD/pCL1925$	50	Not detected	Not detected
MG1655/pTrc99a- budABC/pCL1925KoDD-ddr::T5 chnA ter	50	0.33	Not detected
MG1655 $\Delta yqhCD/pTrc99a$ - budABC/pCL1925KoDD-ddr::T5 chnA ter #1	50	0.23	Not detected
MG1655 $\Delta yqhCD/pTrc99a$ - budABC/pCL1925KoDD-ddr::T5 chnA #2	50	0.19	Not detected
Negative control, MG1655 $\Delta yqhCD/pCL1925$	150	Not detected	Not detected
MG1655/pTrc99a- budABC/pCL1925KoDD-ddr::T5 chnA ter	150	0.41	0.12
MG1655 $\Delta yqhCD/pTrc99a$ - budABC/pCL1925KoDD-ddr::T5 chnA #1	150	0.15	0.46
MG1655 $\Delta yqhCD/pTrc99a$ - budABC/pCL1925KoDD-ddr::T5 chnA #2	150	0.44	0.14
Medium		Not detected	Not detected

^a #1 and #2 represent independent isolates.

EXAMPLE 12

Cloning of Amino:Pyruvate Transaminase (APT)

5 An amino:pyruvate transaminase (APT) from *Vibrio Fluvialis* JS17
was identified by Shin et al. (Appl. Microbiol Biotechnol. (2003) 61:463-
471). The amino acid sequence (SEQ ID NO:122) was found to have
significant homology with ω -amino acid:pyruvate transaminases (Shin and
Kim (*J. Org. Chem.* 67:2848-2853 (2002)). It was shown that the *Vibrio*
10 *Fluvialis* APT has transaminase activity towards acetoin.

For expression of the APT enzyme in *E. coli*, a codon optimized APT coding region (SEQ ID NO:144) was designed using the preferred *E. coli* codons with additional considerations such as codon balance and mRNA stability, and synthesized (by DNA2.0; Redwood City, CA). The coding region DNA fragment was subcloned into the pBAD.HisB vector (Invitrogen) between the NcoI and HindIII sites and the resulting plasmid, hereafter referred to as pBAD.APT1, was transformed into TOP10 cells..

EXAMPLE 13

Characterization of *Vibrio Fluvialis* APT Alanine:Acetoin Aminotransferase

20 Activity

A 5 mL volume of LB broth + 100 μ g/mL ampicillin was inoculated with a fresh colony of TOP10/pBAD:APT1 cells. The culture was incubated at 37 °C for approximately 16 h with shaking (225 rpm). A 300 μ L aliquot of this culture was used to inoculate 300 mL of the same medium, which was incubated at 37 °C with shaking (225 rpm). When the culture reached an OD₆₀₀ of 0.8, L-arabinose was added to a final concentration of 0.2 % (w/v). The culture was incubated for an additional 16 h, then harvested. The cells were washed once with 100 mM potassium phosphate buffer (pH 7.8) and then frozen and stored at -80 °C.

To isolate the enzyme, the cell pellet was thawed and resuspended in 8 mL of 100 mM potassium phosphate buffer (pH 7) containing 0.2 mM ethylenediaminetetraacetate, 1 mM dithiothreitol and 1 tablet of protease inhibitor cocktail (Roche; Indianapolis, IN). The cells were lysed by two

passes through a French pressure cell at 900 psi, and the resulting lysate was clarified by centrifugation for 30 min at $17000 \times g$. Ammonium sulfate was added to 35 % saturation, and the solution was stirred for 30 min at room temperature, at which point precipitated solids were removed by centrifugation (30 min, $17000 \times g$). Additional ammonium sulfate was added to the supernatant to give 55% saturation, and the solution was again stirred for 30 min at room temperature. The precipitated solids were removed by centrifugation (30 min, $17000 \times g$) and then resuspended in 5 mL of 100 mM potassium phosphate buffer (pH 7) containing 10 μ M pyridoxal 5'-phosphate and 1 mM dithiothreitol. This solution was desalted by passage through a PD10 column equilibrated with Buffer A (50 mM bis-tris propane buffer (pH 6) containing 10 μ M pyridoxal 5'-phosphate and 1 mM dithiothreitol). The desalted extract was then loaded onto a 20 mL Q-Fast Flow column pre-equilibrated with Buffer A. APT was eluted with a linear gradient of 0-0.1 M NaCl in Buffer A. The enzyme was detected in eluted fractions by the presence of a protein band of size ~ 50 kD when analyzed by SDS-polyacrylamide gel electrophoresis and by the characteristic absorbance at 418 nm. Fractions containing the enzyme eluted at ~ 0.3 M NaCl. These fractions were pooled to yield a total of 6 mL of a 5.45 mg/mL solution of enzyme, which was $>90\%$ pure, as judged by SDS-polyacrylamide gel electrophoresis.

The alanine:acetoin aminotransferase activity of APT was assayed using a lactic dehydrogenase coupled assay. Reaction mixtures contained 100 mM bis-tris propane (pH 9.0), 10 μ M pyridoxal 5'-phosphate, 0-50 mM acetoin, 0-5 mM L-alanine, 0.14 or 0.28 mg/mL purified enzyme, 200 μ M NADH and 20 U/mL lactic dehydrogenase (Sigma; St. Louis, MO). The reaction was followed by measuring the change in absorbance at 340 nm, indicative of the oxidation of NADH. Under these conditions, the k_{cat}/K_m for acetoin was $10 \text{ M}^{-1} \text{ s}^{-1}$ and that for L-alanine was $400 \text{ M}^{-1} \text{ s}^{-1}$.

The identity of the expected product 3-amino-2-butanol was confirmed by comparison to a synthetic standard. A mixture of (*R,R*)- and (*S,S*)-3-amino-2-butanol was synthesized by the method of Dickey *et al.* [J

Amer Chem Soc 74:944 (1952)]: 5 g of *trans*-2,3-epoxybutane were slowly stirred into 150 mL of cold (4 °C) NH₄OH. The reaction was slowly warmed to room temperature, sealed and stirred at room temperature for an additional 10 days. At this time, excess ammonia and water and residual epoxybutane were removed by rotary evaporation under vacuum at 40 °C. The resulting clear oil (2.9 g) was resuspended in water to a concentration of 10% (w/v). Production of the desired product was confirmed by NMR analysis and comparison of the spectrum to that reported by Levy *et al.* [*Org. Magnetic Resonance* 14:214 (1980)]. A mixture of the corresponding (2*R*,3*S*)- and (2*S*,3*R*)- isomers was produced using the identical method with the exception that the starting material was the *cis*- isomer of 2,3-epoxybutane.

An analytical method for detection of 3-amino-2-butanol was developed based on the *o*-phthaldialdehyde derivatization method for amino acid determination reported by Roth [*Anal. Chem.* 43:880 (1971)]. A 200 µL aliquot of 1 mM 3-amino-2-butanol (mixture of isomers) was mixed with 200 µL of a 50 mM solution of borate (pH 9.5), to which was added 10 µL of 5 µL/mL 2-mercaptoethanol in ethanol and 10 µL of 10 mg/mL *o*-phthaldialdehyde in ethanol. The solution was incubated at room temperature for 10 min, at which time the derivative was extracted into 200 µL hexane. The hexane was separated from the aqueous solution by decanting, and 10 µL were injected onto a Chiracel OD HPLC column (Daicel Chemical Industries; Fort Lee, NJ). The column was run isocratically with a mobile phase of 90:10 hexane:isopropanol at a rate of 1 mL/min. The derivatized isomers of 3-amino-2-butanol were detected by absorbance at 340 nm with retention times of approximately 15.7 and 16.8 min [(2*S*,3*S*) and (2*R*,3*R*)], and 18.4 and 21.9 min [(2*R*,3*S*) and (2*S*,3*R*)]. To differentiate the enantiomers in the first mixture, the pure (2*R*,3*R*) isomer (Bridge Organics; Vicksburg, MI) was also run under the identical conditions and found to be the 16.8 min peak. To differentiate the enantiomers in the second mixture, the mixture was first kinetically resolved using the alanine:acetoin aminotransferase: 0.28 mg of purified enzyme was incubated with 10 mM pyruvate and 10 mM 3-amino-2-

butanol [1:1 mixture of (2*R*,3*S*) and (2*S*,3*R*) isomers] in 1 mL of 100 mM bis-tris propane (pH 9.0). After 24 h at room temperature, an aliquot was removed and analyzed as described above. Analysis revealed that the 18.4 min peak was 95 % depleted, while the 21.9 min peak was >90 % retained. A 100 μ L aliquot of the remaining reaction mixture was mixed with 50 μ L of 20 mM NADH and 10 μ L of extract from the TOP10/pTrc99a-BudC strain described in Example 9. The BudC enzyme is known to reduce (*R*)-acetoin to *meso*-2,3-butanediol and (*S*)-acetoin to (*S,S*)-2,3-butanediol [Ui *et al.* (2004) *Letters in Applied Microbiology* 39:533-537].

After 3 h, samples were taken from the reaction and analyzed as described above for acetoin and butanediol. The analysis indicated that the primary product of the reduction was *meso*-2,3-butanediol, indicating that the product of the aminotransferase reaction was (*R*)-acetoin, and therefore the consumed 3-amino-2-butanol isomer was the (2*R*,3*S*) isomer. Thus the retention time of 18.4 min can be assigned to this isomer and 21.9 to the (2*S*,3*R*) isomer.

To confirm that the product of the APT-catalyzed alanine:acetoin aminotransferase reaction was 3-amino-2-butanol, 0.28 mg of purified enzyme was incubated with 10 mM acetoin, 10 mM L-alanine, 50 U lactic dehydrogenase and 200 μ M NADH in 1 mL of 100 mM bis-tris propane (pH 9.0). The reaction mixture was incubated at room temperature for 20 h, after which a 200 μ L aliquot was removed and derivatized as described above. The retention times of the derivatized products were 15.8 min (major product) and 18.5 min (minor product), matching that of the (2*S*,3*S*)- and (2*R*,3*S*)-3-amino-2-butanol standards.

EXAMPLE 14

Identification and Cloning of *Erwinia carotovora* subsp. *atroseptica* Amino Alcohol Kinase and Amino Alcohol O-Phosphate Lyase

The purpose of this example is to describe the identification and cloning of sequences encoding an amino alcohol kinase and amino alcohol O-phosphate lyase from the bacterium *Erwinia carotovora*. These two enzymes are part of Pathway 1 for the conversion of 3-amino-2-

butanol to 2-butanone *via* the intermediate 3-amino-2-butanol phosphate as shown in Figure 1.

Prediction of the *Erwinia* amino alcohol kinase and the amino alcohol O-phosphate lyase

- 5 ATP-dependent amino alcohol kinase and amino alcohol O-phosphate lyase activities have been detected in several *Pseudomonas* and *Erwinia* species, including *Pseudomonas* sp. P6 (NCIB10431), *Pseudomonas putida* NCIB 10558 (Jones et al. (1973) *Biochem. J.* 134:167-182), *Erwinia carotovora*, *Erwinia amanas*, *Erwinia milletiae*, and
- 10 *Erwinia atroseptica* (Jones et al. (1973) *Biochem. J.* 134:959-968). In these studies, the extracts of the above species were shown to have activity for the enzymatic conversion of aminopropanol through aminopropanol O-phosphate to propionaldehyde, and the conversion of ethanolamine through ethanolamine O-phosphate to acetaldehyde.
- 15 The genomic sequence of the *Erwinia atroseptica* strain in which these activities were reported to exist (now designated as *Erwinia carotovora* subsp. *atroseptica* strain SCRI1043 (ATCC BAA-672)) has been determined at the Sanger Institute (Bell et al. *Proc. Natl. Acad. Sci. USA* 101(30): 11105-11110). Analysis of the putative kinases in the
- 20 *Erwinia carotovora* subsp. *atroseptica* genome revealed an operon sequence (SEQ ID NO: 275) encoding a putative protein (ECA2059; SEQ ID NO:124) that is 39% identical to a *Rhizobium loti* homoserine kinase and a putative class-III pyridoxal phosphate (PLP)-dependent aminotransferase (ECA2060; SEQ ID NO:126) that is 58% identical to a
- 25 putative aminotransferase from *Rhizobium meliloti*. It was expected that ECA2059 was an amino alcohol kinase and ECA2060 was an amino alcohol O-phosphate lyase which uses PLP as cofactor.

Cloning of the putative amino alcohol kinase and putative amino alcohol O-phosphate lyase from *Erwinia carotovora* subsp. *atroseptica*

- 30 Genomic DNA of *Erwinia carotovora* subsp. *atroseptica* (ATCC #: BAA-672D) was obtained from American Type Culture Collection (ATCC). The operon encoding the putative amino alcohol kinase (KA) and amino alcohol O-phosphate lyase (AT) was named KA-AT (SEQ ID NO: 275).

This operon was amplified from the *Erwinia* genomic DNA by Phusion DNA polymerase (Finnzymes; via New England Biolabs; Ipswich, MA) using primers OT872 (SEQ. ID. No. 127) and OT873 (SEQ. ID. No. 128). A DNA fragment of 2.4 kb was obtained by the PCR reaction, which

5 corresponds to the size of the KA-AT operon. The PCR product was digested with EcoRI and PstI restriction enzymes, and cloned into vector pKK223-3 (Amersham Biosciences; Piscataway, NJ) which was digested with the same restriction enzymes. This produced plasmid pKK223.KA-AT, which contained the putative *Erwinia* amino alcohol kinase-lyase

10 operon under control of the *tac* promoter. Similarly, plasmids pKK223.KA and pKK223.AT were made which placed the putative *Erwinia* kinase and the putative *Erwinia* lyase coding regions in separate vectors, each under the control of the *tac* promoter. For the PCR cloning of the KA coding region (SEQ ID NO:123), primers OT872 (SEQ. ID. No. 127) and OT879

15 (SEQ. ID. No. 129) were used; and for the PCR cloning of AT coding region (SEQ ID NO:125), primers OT873 (SEQ. ID. No. 128) and OT880 (SEQ. ID. No. 130) were used in the PCR amplifications, which generated PCR products of 1.1 kb and 1.3 kb respectively. The PCR products were each digested with EcoRI and PstI, and ligated into vector pKK223-3 to

20 generate pKK223.KA and pKK223.AT.

In vivo activity of the putative amino alcohol kinase and putative amino alcohol O-phosphatase lyase from *Erwinia carotovora* subsp. *atroseptica*

Plasmids pKK223.KA-AT, pKK223.KA, pKK223.AT and pKK223-3 were transformed into the *E. coli* MG1655 strain. The transformants were

25 restreaked onto a MOPS minimal media plate containing 1% glucose, 0.5% aminopropanol as a sole nitrogen source, 1 mM IPTG and 100 µg/mL ampicillin. Expression of KA-AT, KA and AT genes were induced by the IPTG. A control plate had no IPTG included. The plates were incubated at 37 °C for 7 days. On the plate with IPTG, only the strain

30 MG1655/pKK223.KA-AT grew, while all the other three strains did not grow. On the plate without added IPTG, the strain MG1655/pKK223.KA-AT grew, but the colonies were significantly smaller than those on the IPTG-containing plate, which corresponds to the lower expression levels

of KA and AT in the uninduced cells. None of the other three strains grew on this plate. This indicates that the co-expression of the putative *Erwinia* KA and AT genes provided sufficient enzyme activities that allowed the *E. coli* strain MG1655/pKK223.KA-AT to utilize aminopropanol as a sole
5 nitrogen source. Expression of each individual enzyme of either KA or AT was not sufficient to provide such enzyme activity *in vivo*.

EXAMPLE 15

In vitro Activity of *Erwinia* putative Amino Alcohol Kinase and Amino Alcohol O-Phosphate Lyase

10 Subcloning of the *Erwinia* KA-AT operon into the pBAD.HisB vector and induction of protein expression

The protein expression levels of *Erwinia* putative KA and AT enzymes expressed in MG1655 cells from the pKK223.KA-AT vector were analyzed by SDS-PAGE analysis. The expression level of the *Erwinia* AT
15 enzyme was relatively low, with a new protein band detected at the correct molecular weight of 46 kD in the soluble fraction of a cell extract, while no new protein band was detected at the size predicted for the KA enzyme.

In an effort to improve the expression of the *Erwinia* putative KA and AT genes, the KA-AT operon was subcloned into the EcoRI and
20 HindIII sites of vector pBAD.HisB-EcoRI. pBAD.HisB-EcoRI was derived from the pBAD.HisB vector (Invitrogen), by replacing the NcoI site in pBAD.HisB with an EcoRI site *via* QuickChange site-directed mutagenesis (Stratagene, La Jolla, CA) using primers OT909 (SEQ ID.# 131) & OT910 (SEQ ID.# 132). In the constructed plasmid pBAD.KA-AT, the KA-AT
25 operon was placed directly under control of the *araB* promoter (without His-tag).

The pBAD.KA-AT plasmid was transformed into the *E. coli* TOP10 strain. A 50 mL culture of TOP10/pBAD.KA-AT strain was grown to mid log phase ($OD_{600}=0.6$) in LB, 100 μ g/mL ampicillin media at 37 °C with
30 shaking at 250 rpm. The culture was induced by addition of L-arabinose to a final concentration of 0.1% (w/v), and it was further incubated at 37 °C for 5 h before harvesting by centrifugation. The cell pellet was resuspended in ice cold 50 mM Tris-HCl, pH 8.0, and disrupted by

sonication on ice with a Fischer Sonic Model 300 Dismembrator (Fischer, Pittsburgh, PA) at 50% power, repeating four cycles of 30 seconds sonication with 60 seconds rest in-between each cycle. Each sonicated sample was centrifuged ($15,000 \times g$, 4 min, 4 °C). Clarified cell free
5 extracts were analyzed for protein expression level and amino alcohol O-phosphate lyase activity.

Chemical synthesis of aminobutanol O-phosphate and aminopropanol O-phosphate

The substrate (*R,R*)-3-amino-2-butanol O-phosphate was
10 synthesized by a method based on that reported by Ferrari and Ferrari (US patent 2730542 [1956]) for phosphoethanolamine: 10 mmol of H_3PO_4 in a 50 % (w/v) aqueous solution was mixed with a 50 % (w/v) solution of (*R,R*)-3-amino-2-butanol (Bridge Organics; Vicksburg, MI) while stirring on ice. After mixing, the solution was slowly warmed to room temperature
15 and then stirred under vacuum and heated to 70 °C. After 1 h at 70 °C, the temperature was slowly increased to 185 °C and maintained there for an additional 2 h. At that time, the reaction was cooled to room temperature and the vacuum released. The remaining material was dissolved in water, and analysis by NMR indicated that 80% of the starting
20 material was converted to product with 20% remaining unreacted. No additional products were observed.

The additional substrates (*2R,3S*)-3-amino-2-butanol O-phosphate and (*2S,3R*)-3-amino-2-butanol O-phosphate were synthesized by the same procedure using a 1:1 mixture of (*2R,3S*)-3-amino-2-butanol and
25 (*2S,3R*)-3-amino-2-butanol (synthesized as described in Example 13) as the starting material. DL -1-amino-2-propanol O-phosphate, (*S*)-2-amino-1-propanol O-phosphate, and (*R*)-2-amino-1-propanol O-phosphate were synthesized by the same procedure using DL-1-amino-2-propanol, (*R*)-2-amino-1-propanol, or (*S*)-2-amino-1-propanol as the starting material.

30 Analysis of the aminopropanol O-phosphate lyase activity encoded by the putative Erwinia KA-AT operon

The aminopropanol O-phosphate lyase assay was performed as described by Jones et al. (1973, *Biochem. J.* 134:167-182) and G. Gori et

al. (1995, Chromatographia 40:336) The formation of propionaldehyde from aminopropanol O-phosphate was assayed colorimetrically with MBTH, which allows the detection of aldehyde formation. The reaction was performed as follows. In a 1 mL reaction, 100 μ g cell free extract of

5 *E. coli* TOP10/pBAD.KA-AT was added to 10 mM DL-1-amino-2-propanol O-phosphate in 100 mM Tris-HCl, pH 7.8, with 0.1 mM PLP. The reaction was incubated at 37 °C for 10 min and 30 min, with an aliquot of 100 μ L reaction mixture removed at each time point and mixed with 100 μ L of 6 mg/mL MBTH in 375 mM glycine-HCl, pH 2.7. This mixture was

10 incubated at 100 °C for 3 min, cooled on ice for 15-30 s, and 1 mL of 3.3 mg/mL FeCl₃.6H₂O (in 10 mM HCl) was added, followed by incubation for 30 min at room temperature. The absorbance of the reaction mixture which contains the aldehyde-MBTH adduct, was measured at 670 nm. The results of the assay are listed in Table 9. In the presence of the

15 aminopropanol phosphate substrate, PLP and cell free extract, formation of aldehyde was detected, as indicated by an Abs₆₇₀ that was higher than the control background of up to 0.3. In the absence of either the substrate or the cell free extract, no aldehyde formation was detected. In the absence of added PLP, somewhat less amount aldehyde was detected,

20 presumably due to the presence of PLP in the cell free extract. Cell free extract of the uninduced TOP10/pBAD.KA-AT- culture did not produce any detectable aldehyde in the reaction. These results indicated that the putative *Erwinia* amino alcohol O-phosphate lyase does catalyze the conversion of aminopropanol O-phosphate to propionaldehyde.

25

Table 9.

Aminopropanol O-phosphate lyase assay. Sample 1 was the cell free extract of a non-induced control of *E. coli* TOP10/pBAD.KA-AT. Samples

2-5 contained the cell free extract of the induced culture *E. coli*

TOP10/pBAD.KA-AT.

30

Sample Number	Induction by 0.1% arabinose	Aminopropanol O-phosphate	PLP	Enzyme extract (100 μ g/mL)	OD ₆₇₀ , 10 min	OD ₆₇₀ , 30 min
1	uninduced	(+)	(+)	(+)	0.262	0.255

2	induced	(+)	(+)	(+)	1.229	2.264
3	induced	(-)	(+)	(+)	0.303	0.223
4	induced	(+)	(-)	(+)	0.855	1.454
5	induced	(+)	(+)	(-)	0.156	0.065

Analysis of the activity of the *Erwinia* amino alcohol O-phosphate lyase towards aminobutanol O-phosphate substrate

The activity of the amino alcohol O-phosphate lyase towards the aminobutanol O-phosphate substrates was studied under the same conditions as described above. The reaction was carried out at 37 °C overnight in a 1 mL reaction that contained 100 µg of cell free extract of *E. coli* TOP10/pBAD.KA-AT, 10 mM aminobutanol O-phosphate (either the mixture of (R,R)+(S,S) or the mixture of (R,S) + (S,R) isomers described in Example 15) in 100 mM Tris-HCl, pH 7.8, with 0.1 mM PLP. An aliquot of 100 µL reaction mixture was removed and the 2-butanone product was detected using the MBTH derivatization method described in the General Methods. The two peaks representing the derivatized 2-butanone isomers were observed. Therefore the *Erwinia* amino alcohol O-phosphate lyase is an aminobutanol phosphate phospho-lyase in addition to an aminopropanol phosphate phospho-lyase.

Analysis of the activity of the *Erwinia* amino alcohol O-phosphate lyase towards stereoisomers of aminopropanol O-phosphate and aminobutanol O-phosphate

The activity of the *Erwinia* amino alcohol O-phosphate lyase towards various stereoisomers of aminopropanol O-phosphate and aminobutanol O-phosphate was studied under the same conditions as described above. In the presence of the *Erwinia* amino alcohol O-phosphate lyase, both (R) and (S)-2-amino-1-propanol O-phosphate were converted to propanone by the enzyme, but the product yield was much higher with the (S) isomer. The enzyme also produced butanone from both mixtures of 3-amino-2-butanol O-phosphate isomers, with a higher product yield found in the reaction containing the (R,S) and (S,R) substrate isomers. Both propanone and butanone products were

derivatized by MBTH, and detected by HPLC as described in General Methods.

Optimization of the gene expression level for the *Erwinia* amino alcohol kinase and amino alcohol O-phosphate lyase

5 In order to improve the expression levels for the *Erwinia* amino alcohol kinase and the amino alcohol O-phosphate lyase in *E. coli*, codon optimized coding regions for both enzymes (named EKA: SEQ ID NO:155 and EAT: SEQ ID NO:156 respectively) were synthesized by DNA2.0 (Redwood City, CA). Each coding region was synthesized with 5' and 3' 10 tails including restriction sites for cloning: EKA has 5' BbsI and 3' EcoRI, HindIII sites; EAT has 5' EcoRI and 3' HindIII sites. The EKA and EAT coding regions were provided from DNA2.0 as plasmids pEKA and pEAT, which were in the pJ51 vector of DNA2.0. The EKA optimized coding region was subcloned by ligating a BbsI and HindIII digested fragment of 15 pEKA into the pBAD.HisB vector between the NcoI and HindIII sites, to generate plasmid pBAD.EKA. In the resulting plasmid the coding region is 5' to the His tag, so a coding region for an N-terminus His₆ tag fused to the *Erwinia* amino alcohol kinase was constructed by performing a QuickChange site-directed mutagenesis reaction using primers SEQ ID 20 NO:157 and SEQ ID NO:158 to generate vector pBAD.His-EKA.

pBAD.His-EKA was transformed into *E. coli* strain *BL21AI* (*F ompT hsdSB (rB⁻ mB⁻) gal dcm araB::T7RNAP-tetA*; Invitrogen) to produce strain *BL21AI/pBAD.HisA-EKA*. A 50 mL culture of *BL21AI/pBAD.HisA-EKA* was grown to mid-log stage (OD₆₀₀=0.6), induced with 0.1% 25 arabinose, and further incubated at 30°C overnight. Cell free extracts were prepared by sonication. The His₆-tagged fusion protein of *Erwinia* amino alcohol kinase was purified using the ProBond™ Purification System (Invitrogen) under non-denaturing purification conditions following the manufacture's instructions.

30 Prophetic Result

The kinase activity of the His₆-tagged *Erwinia* amino alcohol kinase is analyzed by the ADP Quest Assay (DiscoverX, Fremont, CA) following the manufacture's instructions. This is a biochemical assay that measures

the accumulation of ADP, a product of the amino alcohol kinase reaction using either aminopropanol or aminobutanol as substrate. 10 mM substrate is mixed with His₆-tagged *Erwinia* amino alcohol kinase, in 100 mM Tris-HCl, pH 7.8, 10 mM MgCl₂, 2 mM KCl, 0.1 mM ATP, and
 5 incubated at 37 °C for 1 h in a 0.2 mL reaction. ADP reagent A (100 µL) and ADP reagent B (200 µL) are added and the mixture is incubated at room temperature for 30 min. The fluorescence signal indicating activity is measured with excitation wavelength of 530 nm and emission wavelength of 590 nm.

10

EXAMPLE 16Expression of Entire Pathway 3Construction of Vector pCLBudAB-ter-T5chnA

The vector pTrc99a::BudABC (described in Example 9) is digested with EcoRI, and the DNA is treated with Klenow DNA polymerase to blunt
 15 the ends. The blunted vector is subsequently digested with SpeI to yield a 2.5 kb fragment containing the *budA* and *budB* genes. The vector pCL1925-ter-T5chnA (described in Example 9) is digested with HindIII, and the DNA was treated with Klenow DNA polymerase to blunt the ends. The blunted vector is subsequently digested with XbaI to yield a 4.6 kb
 20 fragment which is then ligated to the *budAB* fragment from pTrc99a::BudABC. The resulting plasmid, designated pCLBudAB-ter-T5chnA, is used to transform *E. coli* Top10 cells, and single colonies are screened for proper plasmid structure by PCR using primers pCL1925vecF (SEQ ID NO:62) and N84seqR3 (SEQ ID NO:159).
 25 Plasmid is prepared from a single colony which yields a PCR product of the expected size of 1.4 kb.

Construction of vector pKK223.KA-AT-APT

The APT gene is amplified from the vector pBAD.APT (described in Example 12) by PCR using primers APTfor (SEQ ID NO:162; 5' includes
 30 RBS and SmaI site) and APTrev (SEQ ID NO:163; 3' adds SmaI site). The product of expected size of 1.7 kbp is gel purified and digested with SmaI to yield blunt ends. The vector pKK223.KA-AT (described in Example 14) is digested with PstI, and the DNA is treated with Klenow

DNA polymerase to blunt the ends. The resulting DNA fragment is ligated with the SmaI-digested PCR product, and the ligation product is used to transform *E. coli* Top10 cells. Individual ampicillin resistant colonies are screened by PCR using primers OT872 (SEQ ID NO:127) and APTrev (SEQ ID NO:163). The presence of a PCR product of the expected size of 4.1 kbp indicates that the gene encoding APT is present and oriented in the same direction as the genes encoding KA and AT. The sequence of the insert is verified using the primers APTseqRev (SEQ ID NO:160) and APTseqFor (SEQ ID NO:161). This plasmid is named pKK223.KA-AT-APT. Proper expression of all three genes is verified by growing a 5 mL culture of Top10/pKK223.KA-AT-APT in LB + 100 μ g/mL ampicillin at 37 °C with shaking. When the OD₆₀₀ reaches ~0.8, expression of the genes on the plasmid is induced by addition of IPTG to 0.4 mM. The expression is evaluated by SDS PAGE and activity assays as described above.

15 Construction of 2-butanol production strain and production of 2-butanone and 2-butanol

E. coli strain MG1655 is transformed with both pKK223.KA-AT-APT and pCLBudAB-ter-T5chnA, and transformants selected for ampicillin and spectinomycin resistance, indicative of the presence of the plasmids. The cells are inoculated into shake flasks (approximately 175 mL total volume) containing 50 or 150 mL of TM3a/glucose medium (with appropriate antibiotics) to represent medium and low oxygen conditions, respectively. IPTG is added to 0.4 mM to induce expression of genes from pKK223.KA-AT-APT. As a negative control, MG1655 cells are grown in the same medium lacking antibiotics. The flasks are inoculated at a starting OD₆₀₀ of ≤ 0.01 and incubated at 34 °C with shaking at 300 rpm for 24 h. The flasks containing 50 mL of medium are capped with vented caps; the flasks containing 150 mL are capped with non-vented caps to minimize air exchange. The MG1655/ pKK223.KA-AT-APT/pCLBudAB-ter-T5chnA strain comprising a 2-butanol biosynthetic pathway produces both 2-butanone and 2-butanol under low and medium oxygen conditions while the negative control strain does not produce detectable levels of either 2-butanone or 2-butanol.

EXAMPLE 17

Characterization of Glycerol Dehydratase Butanediol Dehydratase Activity

Glycerol dehydratase (E.C. 4.2.1.30) and diol dehydratase (E.C. 4.2.1.28), while structurally related, are often distinguished in the art based on various differences that include substrate specificity. This example demonstrates that glycerol dehydratase converts *meso*-2,3-butanediol to 2-butanone. The recombinant *E. coli* strain KLP23/pSYCO12, comprising *Klebsiella pneumoniae* genes encoding the multiple subunits of glycerol dehydratase (alpha: SEQ ID NO:145 (coding region) and 146 (protein); beta: SEQ ID NO: 147 (coding region) and 148 (protein); and gamma: SEQ ID NO: 149 (coding region) and 150 (protein)) and *Klebsiella pneumoniae* genes encoding the multiple subunits of glycerol dehydratase reactivase (large subunit, SEQ ID NO: 151 (coding region) and 152 (protein); and small subunit, SEQ ID NO: 153 (coding region) and 154 (protein)), is described in Emptage *et al.* US 6,514,733 and in WO 2003089621, which are herein incorporated by reference. A crude, cell free extract of KLP23/pSYCO12 was prepared by methods known to one skilled in the art. Enzyme assay was performed in the absence of light in 80 mM HEPES buffer, pH 8.2 at 37 °C with 12 μ M coenzyme B₁₂ and 10 mM *meso*-2,3-butanediol. The formation of 2-butanone was monitored by HPLC (Shodex SH-1011 column and SH-G guard column with refractive index detection; 0.01 M H₂SO₄ as the mobile phase at a flow rate of 0.5 mL/min and a column temperature of 50 °C; 2-butanone retention time = 40.2 min). The rate of 2-butanone formation by the glycerol dehydratase preparation was determined to be 0.4 nmol/min/mg of crude protein.

EXAMPLE 18

Structural Analysis of Diol/Glycerol Dehydratases through Generation and

Validation of a Profile HMM for Experimentally Proven Diol/Glycerol

Dehydratases

The diol dehydratase and glycerol dehydratase enzymes belong to the enzyme classes 4.2.1.28 and 4.2.1.30, respectively. The enzymes in both classes are each a complex of three subunits: large (also called

alpha), medium (also called beta) and small (also called gamma). In some glycerol dehydratases the large and medium subunits were found to be fused.

Identifying family members by sequence

5 The *Klebsiella oxytoca* butanediol dehydratase enzyme was used as a prototype enzyme for identifying a family of diol and glycerol dehydratase enzymes. The amino acid sequences of the alpha (GenBank No: BAA08099; SEQ ID NO: 8), beta (GenBank No: BAA08100; SEQ ID NO: 10) and gamma (GenBank No: BAA08101; SEQ ID NO: 12) subunits
10 were each run as the query sequence in a BLASTp search against the GenBank non-redundant protein database using default parameters. Sequences with relevant matches were extracted. Relevance was judged by the E-value score, protein definition, details included in the GenBank report for the matched proteins, and literature review of the topic. For the
15 large subunit, the BLAST output showed an abrupt decrease in the E-value from e-20 to an E-value of 1.5. All sequence matches with an E-value of 1.5 or larger had definitions inconsistent with them being dehydratases. Many of these sequences were labeled as DNA-directed RNA polymerase beta subunits. There were matches with E-values
20 around e-20, which were partial sequences. Sequences with no annotation were included if the E-value was less than 1.5.

 Using the *Klebsiella oxytoca* butanediol dehydratase alpha subunit as a query, 50 homologs were identified as members of this family of proteins. This group included some sequences that were not full length
25 proteins. The full length sequences identified for the alpha subunit family of diol/glycerol dehydratases are the prototype SEQ ID No: 8 and SEQ ID NOs: 93, 99, 105, 135, 138, 141, 146, 164, 167, 170, 173, 176, 179, 182, 185, 188, 191, 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 130, 243, 254, 255, 256, 257, 258, and 259. SEQ ID NOs: 233, 235,
30 237, 239, 241, 246, 247 include both the alpha and beta subunits, which are fused in these cases.

 Using the *Klebsiella oxytoca* butanediol dehydratase beta subunit as a query, 51 homologs were identified as members of this family of

proteins. This group included some sequences that were not full length proteins. The full length sequences identified for the beta subunit family of diol/glycerol dehydratases are the prototype SEQ ID No: 10 and SEQ ID NOs: 95, 101, 107, 136, 139, 142, 148, 165, 168, 171, 174, 177, 180, 183,
 5 186, 189, 192, 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 244, 250, 252, 260, 261, 262, 263, 364, 265, 266, and 167.

Using the *Klebsiella oxytoca* butanediol dehydratase gamma subunit as a query, 48 homologs were identified as members of this family of proteins. This group included some sequences that were not full length
 10 proteins. The full length sequences identified for the gamma subunit family of diol/glycerol dehydratases are the prototype SEQ ID No: 12 and SEQ ID NOs: 97, 103, 109, 137, 140, 143, 150, 166, 169, 172, 175, 178, 181, 184, 187, 190, 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 234, 236, 238, 240, 242, 245, 248, 249, 251, 253, 268, 270,
 15 271, 272, 273, and 274.

Identifying family members with experimentally assessed function

For each sequence identified through the analysis described above, a search for experimental evidence of its biochemical function was conducted in the BRENDA, UniProt and NCBI Entrez databases.
 20 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 UniProt Knowledgebase is composed of a human-curated part the Swiss-Prot database and of a
 25 machine annotated supplement the TrEMBL database. The curated Swiss-Prot database (Swiss Institute of Bioinformatics) provides a high level of protein annotation that includes domain architecture, post-translational modifications and sequence variants. NCBI Entrez is the integrated, text-based search and retrieval system used at NCBI (National
 30 Center for Biotechnology Information, Bethesda, MD) for the major databases, including PubMed, Nucleotide and Protein Sequences, Protein Structures, Complete Genomes, and Taxonomy.

Through the analysis of information and references identified from these databases, eight diol/glycerol dehydratases with experimentally verified function as a diol or glycerol dehydratase were identified. These are given in Table 10.

5

Table 10.Diol/glycerol dehydratases with experimentally verified function

organism	subunit	Genbank #	SEQ ID No	type	reference
Klebsiella oxytoca	large	gi 6980836	8	diol	Shibata et al., Structure 1999; 7:997-1008
K. oxytoca	medium	gi 6980837	10	diol	
K. oxytoca	small	gi 6980838	12	diol	
Klebsiella pneumoniae	large	gi 4063702	105	diol	Tobimatsu et al., 1998; Biosci. Biotechnol. Biochem. 62:1774-1777
K. pneumoniae	medium	gi 94470233	107	diol	
K. pneumoniae	small	gi 4063704	109	diol	
Clostridium pasteurianum	large	gi 3360389	135	glycerol	Macis et al., FEMS Microbiol Lett. 1998; 164(1):21-8
C.pasteurianum	medium	gi 3360390	136	glycerol	
C.pasteurianum	small	gi 3360391	137	glycerol	
Escherichia blattae	large	gi 60099613	138	glycerol	Sönke et al. J. of Mol. Micro. and Biotech. 2004; 8:150-168
E.blattae	medium	gi 57340191	139	glycerol	
E.blattae	small	gi 57340192	140	glycerol	
Klebsiella pneumoniae	large	gi 24158719	146	glycerol	Willard, Thesis (1994), U of Wisconsin-Madison
K. pneumoniae	medium	gi 24158720	148	glycerol	
K. pneumoniae	small	gi 24158721	150	glycerol	
Citrobacter freundii	large	gi 1169287	141	glycerol	Seyfried, Gottschalk; J. Bacteriol. 178:5793-5796 (1996)
C. freundii	medium	gi 1229154	142	glycerol	
C. freundii	small	gi 1229155	143	glycerol	
Lactobacillus brevis	large	gi 116334196	164	diol	Schuetz and Radler 1984; Arch. Microbiol. 139, 366-370
L.brevis	medium	gi 116334195	165	diol	

L.brevis	small	gi 116334194	166	diol	
Lactobacillus collinoides	large	gi 18857678	99	diol	Sauvageot et al., 2002; Eur J Biochem. 269(22):5731-7.
L.collinoides	medium	gi 18857679	101	diol	
L.collinoides	small	gi 18857680	103	diol	

The set of 8 amino acid sequences of each subunit from diol/glycerol dehydratases with experimentally determined function, listed in Table 10, were compared by making a multiple sequence alignment using ClustalW with default parameters. The % identity for the large subunit sequences ranged from 97.6% to 58.4%. The % identity for the medium subunit sequences ranged from 89.5% to 41.7%. The % identity for the small subunit sequences ranged from 83.3% to 36.4%. Thus the amount of sequence identity between some subunit sequences was very low (such as 36.4%, 41.7%) even though these subunits were known to be components of enzymes known through experimental data to perform the same function. The low level of % sequence identity made it impractical to use this criterion for structure/function correlations

Sequence Relationship of Experimentally Verified Diol/Glyderol Dehydratases to Other Diol/Glycerol Dehydratases

To perform this analysis, highly redundant sequences that are >95% identical were removed from the sequence set for large, medium or small subunits, except that all experimentally verified function sequences were retained. Truncated or partial protein sequences were also removed. A multiple sequence alignment was performed on the remaining sequences using ClustalW with default parameters. The % identity for the large subunits ranged from 97.6% (highest % is from multiple experimentally verified sequences) to 42.8 %. The % identity for the medium subunits ranged from 91.9% to 26.4%. The % identity for the small subunits ranged from 85.2% to 20.5%. These % identities are similar ranges to the % identities for the experimentally verified sequences.

Based on the multiple sequence alignments, phylogenetic trees were built using the neighbor-joining algorithm (as implemented in the MEGA software version 3.1 package; Kumar et al., 2004 Briefings in Bioinformatics 5:150-163.) The phylogenetic trees are shown in Figures 2 (large subunit), 3 (medium subunit), and 4 (small subunit), with the identities of the mapped sequences listed in a key for each figure. As seen from the positions marked for the experimentally verified function sequences (in dark and light grey circles for diol dehydratase and glycerol dehydratase, respectively), these sequences are spread over most of the tree. However, each tree does include a branch with no experimentally verified members, but which appears to belong to the diol/glycerol dehydratase family.

Building a Profile Hidden Markov Model (HMM) of the diol/glycerol-dehydratase family based on the sets of eight subunit sequences

An alternative structure/function characterization of the sets of subunits of the diol/glycerol dehydratase family of enzymes was performed using the HMMER software package (The theory behind profile HMMs is described in R. Durbin, S. Eddy, A. Krogh, and G. Mitchison, Biological sequence analysis: probabilistic models of proteins and nucleic acids, Cambridge University Press, 1998; Krogh et al., 1994; J. Mol. Biol. 235:1501-1531), following the user guide which is available from HMMER (Janelia Farm Research Campus, Ashburn, VA).

Each set of 8 amino acid sequences for the large, medium, and small subunits of functionally characterized diol/glycerol dehydratases (in Table 10) was separately analyzed using the HMMER software program. The output of the HMMER software program is a Profile Hidden Markov Model (HMM) that characterizes the input sequences. As stated in the user guide, Profile HMMs are statistical models of multiple sequence alignments. They capture position-specific information about how conserved each column of the alignment is, and which amino acid residues are most likely to occur at each position. Thus HMMs have a formal probabilistic basis. Profile HMMs for a large number of protein

families are publicly available in the PFAM database (Janelia Farm Research Campus, Ashburn, VA).

Each Profile HMM was built as follows:

Step 1. Build a sequence alignment

5 The eight sequences for the large subunit of the functionally verified diol/glycerol dehydratases (SEQ ID NOs:8, 99, 105, 135, 138, 141, 146, and 164) were aligned using Clustal W with default parameters. The same was done for the set of medium subunit sequences (SEQ ID NOs:10, 101, 107, 136, 139, 142, 148, and 165) and the set of small
10 subunit sequences (SEQ ID NOs:12, 103, 109, 137, 140, 143, 150 and 166).

Step 2. Build a Profile HMM

 The hmmbuild program was run on each set of the aligned sequences using default parameters. hmmbuild reads the multiple
15 sequence alignment file, builds a new Profile HMM, and saves the Profile HMM to file. Using this program an un-calibrated profile was generated from the multiple alignment for each set of subunit sequences described above.

 The following information based on the HMMER software user
20 guide gives some description of the way that the hmmbuild program prepares a Profile HMM. A Profile HMM is capable of modeling gapped alignments, e.g. including insertions and deletions, which lets the software describe a complete conserved domain (rather than just a small ungapped motif). Insertions and deletions are modeled using insertion (I) states and
25 deletion (D) states. All columns that contain more than a certain fraction x of gap characters will be assigned as an insert column. By default, x is set to 0.5. Each match state has an I and a D state associated with it. HMMER calls a group of three states (M/D/I) at the same consensus position in the alignment a "node". These states are interconnected with
30 arrows called *state transition probabilities*. M and I states are emitters, while D states are silent. The transitions are arranged so that at each node, either the M state is used (and a residue is aligned and scored) or the D state is used (and no residue is aligned, resulting in a deletion-gap

character, '-'). Insertions occur between nodes, and I states have a self-transition, allowing one or more inserted residues to occur between consensus columns.

The scores of residues in a match state (i.e. match state emission scores), or in an insert state (i.e. insert state emission scores) are proportional to $\text{Log}_2(p_x / (\text{null}_x))$. Where p_x is the probability of an amino acid residue, at a particular position in the alignment, according to the Profile HMM and null_x is the probability according to the Null model. The Null model is a simple one state probabilistic model with pre-calculated set of emission probabilities for each of the 20 amino acids derived from the distribution of amino acids in the SWISSPROT release 24.

State transition scores are also calculated as log odds parameters and are proportional to $\text{Log}_2(t_x)$. Where t_x is the probability of transiting to an emitter or non-emitter state.

Step 3. Calibrate the Profile HMM

Each Profile HMM was read using `hmmcalibrate` which scores a large number of synthesized random sequences with the Profile (the default number of synthetic sequences used is 5,000), fits an extreme value distribution (EVD) to the histogram of those scores, and re-saves the HMM file now including the EVD parameters. These EVD parameters (μ and λ) are used to calculate the E-values of bit scores when the profile is searched against a protein sequence database. `hmmcalibrate` writes two parameters into the HMM file on a line labeled "EVD": these parameters are the μ (location) and λ (scale) parameters of an extreme value distribution (EVD) that best fits a histogram of scores calculated on randomly generated sequences of about the same length and residue composition as SWISS-PROT. This calibration was done once for each Profile HMM.

The calibrated Profiles HMM for the large subunit, medium subunit, and small subunit sets of sequences are provided in the appendix as alpha Profile HMM, beta Profile HMM, and gamma Profile HMM Excel

charts. Each Profile HMM is provided in a chart that gives the probability of each amino acid occurring at each position in the amino acid sequence. The highest probability is highlighted for each position. Table 11 shows a few lines of the Profile HMM prepared for the large subunits of diol/glycerol dehydratases with function experimentally verified.

Table 11.

A portion of the large subunit Profile HMM.

HMM	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
	m->m	m->i	m->d	i->m	i->i	d->m	d->d	b->m	m->e											
	-585	*	-1585																	
1(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	4141	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	-585	*											
2(K)	-603	-1732	-469	811	-2182	-1397	205	-1770	1954	-1654	-860	-59	-1465	629	1868	-495	-471	-1439	-1719	-1294
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-350	-6045	-2321	-894	-1115	-701	-1378	*	*											
3(R)	-781	-1268	-994	-512	-1638	-1313	-151	-1479	778	-1459	-913	-539	-1557	136	3077	-831	-760	-1263	-1437	-1152
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-41	-5736	-6778	-894	-1115	-535	-1690	*	*											

- 10 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.

15 Table 11 shows that for the large subunits, methionine has a 4141 probability of being in the first position, the highest probability which is highlighted. In the second position lysine has the highest probability, which is 1954. In the third position arginine has the highest probability, which is 3077.

Step 4. Test the specificity and sensitivity of the built Profile HMMs

Each Profile HMM was evaluated using hmmsearch, which reads a Profile HMM from hmmfile and searches a sequence file for significantly similar sequence matches. The sequence file searched was the GenBank non-redundant protein database. The size of the database (Z parameter) was set to 1 billion. This size setting ensures that significant E-values against the current database will remain significant in the foreseeable future. The E-value cutoff was set at 10.

The Profile HMMs for the large, medium and small subunits of diol/glycerol dehydratases with experimentally verified function were specific in that only diol/glycerol dehydratase subunits were recovered, as indicated by the annotation of the matched sequences, and sensitive in that even partial sequences of diol/glycerol dehydratase subunits were recovered. Each of the recovered sequences had an E-value of 0.01 or less.

All of the sequences on the phylogenetic trees in Figures 2, 3, and 4 were recovered in the Profile HMM matching. All sequences in the branches of the trees which contain no sequence with experimentally verified function were matched. Thus all of the diol and glycerol dehydratases were linked to the 8 diol and glycerol dehydratases with experimentally verified function through matching with the Profile HMMs for the large, medium, or small subunits of the enzymes. The full length diol and glycerol dehydratase subunits that match the Profile HMMs have the following SEQ ID NOs:

Large (alpha) subunits: 8, 93, 99, 105, 135, 138, 141, 146, 164, 167, 170, 173, 176, 179, 182, 185, 188, 191, 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 130, 243, 254, 255, 256, 257, 258, and 259.

Large + medium subunits fused (large subunit and medium subunit portion match the large Profile and medium Profile, respectively): 233, 235, 237, 239, 241, 246, and 247.

Medium (beta) subunits: 10, 95, 101, 107, 136, 139, 142, 148, 165, 168, 171, 174, 177, 180, 183, 186, 189, 192, 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 244, 250, 252, 260, 261, 262, 263, 364, 265, 266, and 167.

Small (gamma) subunits: 12, 97, 103, 109, 137, 140, 143, 150, 166, 169, 172, 175, 178, 181, 184, 187, 190, 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 234, 236, 238, 240, 242, 245, 248, 249, 251, 253, 268, 270, 271, 272, 273, and 274.

This analysis shows that the Profile HMM for each subunit, that was prepared using sequences with experimentally verified function, provides a structure that is linked to function of diol/glycerol dehydratase enzymes.

Matching of all of the above sequences to the Profiles HMM in turn provides a structure/function link for these sequences.

TABLE 12

HMMER2.0 [2.3.2]		Program name and version																			
NAME alpha_exp_seqs		Name of the input sequence alignment file																			
LENG 557		Length of the alignment: include indels																			
ALPH Amino		Type of residues																			
MAP yes		Map of the match states to the columns of the alignment																			
COM hmmbuild alpha.hmm alpha_exp_seqs.aln		Commands used to generate the file: this one means that hmmbuild (default parameters) was applied to the alignment file																			
COM hmmscalibrate alpha.hmm		Commands used to generate the file: this one means that hmmscalibrate (default parameters) was applied to the hmm profile																			
NSEQ 8		Number of sequences in the alignment file																			
DATE Fri Mar 30 19:02:15 2007		When was the file generated																			
XT -8455 -4 -1000 -1000 -8455 -4 -8455 -4		The transition probability distribution for the null model (single G state).																			
NULT -4 -8455		The symbol emission probability distribution for the null model (G state); (e.g. 4 or 20) integers. The null probability used to convert these back to model probabilities is 1/K.																			
NULE 595 -1558 85 338 -294 453 -1158 197 249 902 -1085 -142 -21 -313 45 531 201 384 -1998 -644																					
EVD -264.989197 0.112643		The extreme value distribution parameters μ and λ respectively; both floating point values. λ is positive and nonzero. These values are set when the model is calibrated with hmmscalibrate.																			
HMM	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	
	m->m	m->i	m->d	i->m	i->i	d->m	d->d	b->m	m->e												
	-585	*	-1585																		
1(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	-1411	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	-585	*												
2(K)	-603	-1732	-469	811	-2182	-1397	205	-1770	-1954	-1654	-860	-59	-1465	629	1868	-495	-471	-1439	-1719	-1294	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-350	-6045	-2321	-894	-1115	-701	-1378	*	*												

3(R)	-781	-1268	-994	-512	-1638	-1313	-151	-1479	778	-1459	-913	-539	-1557	136	3077	-831	-760	-1263	-1437	-1152	3
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5736	-6778	-894	-1115	-535	-1690	*	*												
4(Q)	-149	-1077	25	226	-1650	-945	-85	-1408	309	-1505	-735	33	-1280	1816	-34	1644	-225	-1020	-1731	-1155	4
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
5(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	3103	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528	5
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
6(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1857	-1326	-899	-1873	-156	3432	-1225	-1148	-1717	-1746	-1529	6
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
7(F)	-1602	-1340	-2512	-2455	5740	-2315	-379	-478	-2225	-175	-180	-1874	-2503	-1781	-2079	-1892	-1647	-683	210	1257	7
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
8(E)	37	-1709	562	2097	-2000	-1013	65	-1701	388	-1724	-924	314	-1290	1204	-72	-263	-376	-1331	-1960	-1293	8
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
9(V)	428	-898	-212	744	-1063	-1160	90	-556	881	-841	-94	22	-1284	414	20	-168	-65	5412	-1268	-753	9
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
10(L)	-1368	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	10
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
11(E)	995	-1189	274	1599	-1710	-927	-3	-1362	257	-1488	-682	191	-1227	366	-212	521	-177	-986	-1785	-1178	11
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

12(K)	-509	-1745	1	892	-2124	-1242	174	-1769	2128	-1689	-880	106	-1385	1310	676	-383	416	-1413	-1802	-1283	12
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
13(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1844	559	-1867	-1326	-899	-1873	-156	3432	-1225	-1148	-1717	-1746	-1529	13
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
14(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	3760	-1471	-1571	-1129	-1216	-1780	-2050	-2023	14
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
15(V)	-886	-604	-2944	-2505	-743	-2731	-2045	1944	-2256	229	340	-2308	-2716	-2086	-2330	-1950	-884	2675	-1910	-1506	15
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
16(H)	-457	-1514	304	388	-1218	-1098	2418	-1597	328	-1620	-886	2258	-1387	298	-36	-374	-457	-1264	-1448	-664	16
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
17(Q)	-346	-1181	-403	128	-1300	-1307	121	-906	1166	365	-294	-69	-1397	1890	525	-356	-260	-706	-1373	-890	17
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
18(D)	-1130	-2063	3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	18
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
19(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	19

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

20(F)	-1300	-1041	-2794	-2396	2872	-2678	-676	440	-2076	1465	808	-1982	-2569	-1598	-1976	-1862	-1239	98	-109	791	20
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

21(V)	-922	-605	-3064	-2618	-752	-2841	-2139	2359	-2383	244	349	-2414	-2784	-2191	-2442	-2058	-911	2430	-1948	-1560	21
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

22(K)	-395	-1326	-130	117	-1960	1239	-86	-1633	2073	-1681	-915	-74	-1403	278	289	-375	-423	-1258	-1820	-1352	22
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

23(E)	-597	-1990	829	2406	-2276	-963	-75	-2069	89	-2078	-1318	1286	-1352	285	-438	-411	-615	-1663	-2255	-1512	23
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

24(W)	-1926	-1577	-2416	-2370	192	-2070	-795	-1602	-1882	-1347	-1210	-2018	-2383	-1854	-1777	-2150	-1992	-1643	5696	560	24
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

25(P)	-136	-963	748	291	-1174	-1080	-2	367	217	-956	-244	51	1547	318	-221	-177	-129	-438	-1415	-860	25
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

26(E)	-704	-2274	1609	2494	-2517	-929	-118	-2327	-117	-2302	-1553	440	-1360	238	-777	-462	-729	-1891	-2491	-1671	26
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

27(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	27
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*
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28(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	28
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

29(F)	-1300	-1032	-2939	-2479	-2085	-2797	-1060	610	-2170	2072	1034	-2173	-2620	-1672	-2063	-1972	-1227	200	-496	232	29
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

30(I)	-932	-616	-3069	-2623	-730	-2845	-2140	2586	-2385	279	372	-2420	-2786	-2188	-2440	-2065	-921	2194	-1936	-1554	30
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

31(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	31
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

32(M)	-1097	-948	-2513	-2098	66	-2604	-1439	782	-1681	1487	3575	-2007	-2489	-1444	-1696	-1772	-1045	428	-1173	-886	32
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

33(E)	-103	-1383	474	1130	-1629	-475	238	-1330	588	-1372	919	886	-1165	659	86	417	-51	-957	-1624	-981	33
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

34(S)	317	-370	-658	-616	-1967	1419	-807	-1713	-760	-1924	-1109	-411	-1154	-888	-1027	-2116	-11	-1006	-2151	-1737	34
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

35(P)	-405	-1216	-191	6	-1782	-1120	-188	-1451	1187	-1561	-850	-164	2497	138	133	-424	-456	-1124	-1757	-1301	35
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

36(N)	-556	-847	-1097	-603	1232	-1683	201	-508	-426	-601	46	-1740	-699	-699	-493	-395	1687	1610	36
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*										

37(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	37
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

38(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	-1471	-1571	-1129	-1216	-1780	-2050	-2023	38	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

39(K)	175	-1085	-247	253	-1457	-1107	109	-1024	-1182	-399	40	-300	486	284	-143	-116	-221	-1500	-977	39	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

40(P)	213	-475	-556	-468	-1769	-628	-656	-1500	-525	-1718	-950	-359	-426	-800	1259	-70	-926	-1971	-1515	40	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

41(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	-220	-1038	-1992	-1530	41	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

42(I)	-935	-625	-3052	-2609	-708	-2832	-2125	-2364	307	391	-2408	-2777	-2168	-2419	-2052	-926	2060	-1920	-1540	42	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

43(K)	-489	-1476	-535	74	-1899	-1347	163	-1474	-1467	-693	-94	-1441	566	1161	-436	566	-1175	-1619	-1191	43	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

44(I)	-935	-625	-3052	-2609	-708	-2832	-2125	2677	-2364	307	391	-2408	-2777	-2168	-2419	-2052	-926	2060	-1920	-1540	44
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
45(E)	-190	-1482	610	1617	-1728	-1033	193	-1411	949	-1462	-616	305	-1203	607	49	-87	-141	-15	-1713	-1064	45
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
46(N)	-508	-1893	708	1172	-2155	-1000	25	-1908	309	-1897	-1104	2413	-1320	976	-163	-338	-492	-1519	-2096	-1394	46
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
47(G)	-758	-1103	-1313	-1487	-2460	333	-1582	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	47
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
48(K)	398	-959	-358	223	-1120	-1205	148	-688	1297	-524	-121	6	-1289	492	847	-176	-70	241	-1260	-760	48
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
49(V)	-905	-603	-3012	-2567	-752	-2784	-2098	2117	-2327	233	342	-2367	-2755	-2145	-2394	-2012	-898	2593	-1933	-1537	49
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
50(V)	-367	-384	-1856	-1345	-565	-1819	-964	1486	-1115	20	389	-1247	-1954	-988	-1285	-941	1596	1684	-1282	-891	50
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
51(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	51
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

52(M)	-1262	-1030	-2945	-2401	126	-2872	-1670	808	-2031	2152	2757	-2305	-2645	-1652	-1984	-2035	-1186	370	-1224	-1041	52
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
53(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	53
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
54(G)	291	-380	-629	-669	-2010	-2409	-887	-1774	-878	-2000	-1199	-445	-1175	-694	-1118	1047	-53	-1050	-2199	-1795	54
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
55(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528	-249	55
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
56(K)	193	-1311	14	503	-1606	-1067	229	-1289	-1052	-1334	-482	797	710	647	530	219	-57	-935	-1580	-977	56
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
57(R)	381	-1078	-400	189	-1317	-1223	150	-885	1141	-1041	-273	-15	-1318	514	-1334	-224	-141	222	-1367	-879	57
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
58(A)	-1262	-1516	1112	1074	-1844	-963	80	-1548	308	-1606	-781	326	-1230	469	-228	238	-247	-1170	-1872	-1200	58
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
59(D)	-668	-2298	-2567	1387	-2516	-816	-71	-2332	-49	-2279	-1509	475	-1334	882	-709	-420	-683	-1885	-2484	-1642	59

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
60(F)	-1602	-1340	-2512	-2455	3740	-2315	-379	-478	-2225	-175	-190	-1874	-2503	-1781	-2079	-1892	-1647	-683	210	1257	60
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
61(D)	-1130	-2063	3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	61
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
62(M)	-1241	-1016	-2914	-2371	122	-2844	-1644	806	-1999	2066	2832	-2273	-2626	-1630	-1957	-2005	-1167	376	-1215	-1025	62
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
63(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698	-1261	63
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
64(D)	-1130	-2063	3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	64
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
65(R)	-439	-1400	-571	103	-1664	-1368	1527	-1291	1414	-314	-534	-77	-1418	594	1819	-398	-324	-1021	-1489	-1036	65
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
66(F)	-1902	-1421	-2805	-2692	3214	-2763	351	-984	-2354	-657	-540	-1668	-2714	-1627	-2106	-1950	-1820	-1082	1026	3156	66
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
67(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698	-1261	67
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*
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68(A)	2806	444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	68
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-359	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

69(D)	196	-1649	1051	-1949	-1018	231	-1691	1047	-1647	-758	919	-1183	668	786	-60	-144	-1263	-1826	-1142	69
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-359	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

70(Y)	1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	4149	70
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-359	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

71(G)	1432	-310	-885	-866	-1952	-2267	-962	-1556	-974	-1871	-1097	-548	-1176	-789	-1180	127	-20	-901	-2164	-1815	71
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-359	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

72(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	-3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698	-1261	72
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-359	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

73(N)	-504	-1608	307	389	-1979	-1097	-14	-1734	1180	-1761	-1004	-1393	350	215	409	-498	-1377	-1890	-1303	73
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-359	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

74(L)	-1081	-806	-3000	-2464	-132	-2843	-1790	1457	-2184	966	-2321	-2664	-1813	-2161	-2002	-1025	1443	-1417	-1198	74
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-359	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

75(E)	88	-1806	1193	-2082	-964	125	-1841	365	-1812	-954	1330	-322	537	-200	-160	-295	-1412	-2017	-1284	75
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-359	-294	-249

[illegible]

83(M)	-491	-492	-1884	-1331	-96	-1923	-814	701	-1026	1207	-1254	-1949	-845	-1137	-1009	548	576	-949	-635	83
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

84(D)	-824	-2383	-2964	1142	-2628	-923	-210	-2461	-314	-2455	-1761	430	-1405	123	-1021	-562	-876	-2027	-2629	-1790	84
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

85(S)	1394	-260	-956	-822	-1809	-507	-852	-1408	-809	-1720	-938	-503	-1137	-658	-1033	-2235	57	-788	-2055	-1662	85
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

86(V)	-466	-415	-2075	-1571	-506	-1975	-1108	1171	-1302	688	460	-1436	-2079	-1152	-1427	-1111	573	-1304	-923	86	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

87(K)	-401	-1577	34	922	-1932	-1184	156	-1558	-2107	-1563	-758	127	-1342	565	523	-304	237	-1220	-1742	-1203	87
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

88(I)	-1120	-818	-3064	-2567	-204	-2914	-1888	-2467	-2246	1556	866	-2411	-2743	-1931	-2224	-2108	-1072	1115	-1497	-1212	88
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

89(A)	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	89	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

90(N)	-477	-1612	-233	247	-2000	-1284	183	-1637	1365	-1583	-780	-1397	598	1526	-382	-381	-1301	-1701	-1216	90	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
91(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	4141	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880	91
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
92(L)	-1306	-1067	-2981	-2443	125	-2920	-1717	814	-2062	2337	2190	-2353	-2682	-1686	-2014	-2092	-1231	388	-1248	-1063	92
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
93(C)	-206	3367	-2319	-1919	-689	-1494	-1270	951	-1603	-153	201	-1455	-1861	-1411	-1625	-746	-346	2000	-1422	-1027	93
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
94(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	94
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
95(I)	-274	-522	-1274	-906	-613	-1430	-689	1794	-672	-125	232	-863	1767	-614	-872	-631	-339	493	-1235	-798	95
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
96(N)	-479	-1529	346	390	-1262	-1084	1651	-1642	280	-1666	-940	2606	-1396	262	-86	-394	-490	-1304	-1493	-708	96
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
97(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1388	-823	2927	-1816	-1362	97
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
98(S)	-161	-1453	838	607	-1778	-999	177	-1491	1000	-1517	-659	304	145	593	16	1073	-129	-1102	-1752	-1097	98

-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
99(R)	-1181	-1614	-1431	-903	-2066	-1632	446	-1944	559	-1867	-1326	-899	-1873	-156	-3452	-1225	-1148	-1717	-1746	-1529
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
100(K)	639	-1359	130	937	-1666	-1054	189	-1344	1343	-1395	-554	234	-1211	599	172	487	-116	-990	-1644	-1036
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
101(E)	-438	-1909	1578	-1930	-2183	-946	37	-1928	199	-1929	-1110	415	-1271	427	-390	-250	509	-1512	-2154	-1401
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
102(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	-3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698	-1261
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
103(I)	-933	-617	-3067	-2622	-727	-2844	-2139	-2601	-2382	283	375	-2419	-2785	-2186	-2438	-2063	-922	2174	-1934	-1553
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
104(E)	375	-1460	295	-1605	-1753	-1028	168	-1444	819	-1486	-844	282	190	577	63	-106	-163	-1079	-1731	-1094
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
105(I)	-486	-338	-2295	-1717	49	-2053	-856	-1705	-1424	1267	888	-1489	-2030	-1121	-1416	-1138	-425	1093	-752	1040
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

106(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	106
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
107(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	107
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
108(A)	-2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	108
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
109(M)	-967	-770	-2707	-2179	-75	-2590	-1515	1740	-1841	1002	-3350	-2043	-2485	-1555	-1846	-1742	-919	818	-1266	-1006	109
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
110(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	110
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
111(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	-3760	-1471	-1571	-1129	-1216	-1780	-2050	-2023	111
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
112(A)	-2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	112
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
113(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	3103	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528	113

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
114(I)	1297	-396	-1045	-533	-555	-1309	-316	1303	-228	-265	313	-551	-1491	-201	106	-396	-102	374	-1004	-589
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
115(V)	433	-490	-819	416	-811	-1396	-259	391	-179	-21	288	-448	-1513	-95	-488	-437	-123	1568	-1055	-615
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
116(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
117(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	2927	-1816	-1362
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
118(M)	-825	-553	-2869	-2332	-347	-2619	-1636	2067	-2072	614	2201	-2123	-2524	-1766	-2066	-1762	-779	1949	-1423	-1130
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
119(S)	1087	-281	-851	-685	-1876	620	-774	-1551	-718	-1778	-951	-428	-1112	-546	-989	2116	79	-874	-2078	-1684
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
120(H)	-303	-1577	31	461	-1878	-1155	1852	-1575	1230	-1543	-699	858	-1278	1692	489	-194	-226	-1208	-1701	-1118
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

121(M)	-1179	-981	-2799	-2262	103	-2750	-1560	800	-1878	1813	3295	-2167	-2570	-1557	-1859	-1910	-1112	400	-1195	-971	121
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
122(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327	122
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
123(V)	-729	-571	-2495	-2034	1817	-2246	-1008	992	-1765	499	636	-1745	-2283	-1457	-1764	-1410	-714	2176	-737	-57	123
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
124(V)	1219	-338	-1791	-1461	-919	-1309	-1142	796	-1253	-361	23	-1160	-1724	-1114	-1391	-557	-284	2221	-1608	-1213	124
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
125(E)	-988	-1921	379	3052	2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	125
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
126(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	4141	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880	126
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
127(M)	-964	-751	-2752	-2222	-95	-2613	-1544	1928	-1891	980	5191	-2075	-2502	-1594	-1889	-1765	-914	876	-1283	-1026	127
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
128(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	4141	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880	128

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
129(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
130(M)	-415	-454	-1730	-1175	-135	-1824	-711	663	-895	1350	-1822	-1122	-1866	-726	-1035	-898	1122	573	-924	-593
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
131(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1660	-1161	-427	-1678	-5540	-59	-855	-904	-1495	-1728	-1237
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
132(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528	132
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
133(M)	-985	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880	133
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
134(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	-3432	-1225	-1148	-1717	-1746	-1529
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
135(A)	-367	-849	-680	-1659	-627	-739	-1190	-650	-1511	-785	-478	1606	-534	-899	74	-21	-698	-1911	-1515	135
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

136(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	-1225	-1148	-1717	-1746	-1529	136
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
137(R)	-957	-1769	-1130	-344	-2309	-1642	117	-1834	1694	-1695	-991	-391	-1689	518	-887	-779	-1577	-1692	-1419	137
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
138(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	-557	-1898	-1539	138
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
139(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	-13760	-1471	-1571	-1129	-1216	-1780	-2050	-2023
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
140(S)	752	-403	-804	-361	-945	-914	-314	-429	-202	-754	1493	-350	-1267	-115	-505	21	-179	-1285	-846	140
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
141(N)	-187	-1264	170	440	-1640	-1019	83	-1319	462	-1408	-602	-1650	-1249	1233	52	-142	1136	-974	-1676	-1083
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
142(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1660	-1161	-427	-1678	-3540	-59	-855	-904	-1495	-1728	-1237
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
143(C)	862	-1939	-1540	-778	-1030	-977	437	-1267	438	89	-1051	-1495	-1057	-1327	-271	-71	1212	-1336	-978	143

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
144(H)	-1192	-1547	-812	-781	-520	-1557	-4586	-1881	-457	-1789	-1357	-866	-1913	-669	-600	-1231	-1258	-1678	-918	-90
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
145(V)	635	-386	-1965	-1657	-921	-1492	-1321	997	-1438	-274	47	-1346	-1881	-1309	-1555	-752	-394	-2522	-1700	-1297
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
146(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	-3069	-557	-1898	-1539
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
147(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	-3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
148(I)	-581	-428	-2332	-1778	-276	-2205	-1155	-1757	-1518	1333	715	-1623	-2188	-1273	-1579	-1307	519	1349	-1155	-831
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
149(K)	-594	-1483	-781	-58	-1796	-1470	168	-1358	-2205	-248	-622	-193	-1513	562	1482	-556	-460	-1115	-1533	-1139
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
150(D)	-1130	-2063	-3241	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

151(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327	151
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
152(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	3760	-1471	-1571	-1129	-1216	-1780	-2050	-2023	152
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
153(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	2927	-1816	-1362	153
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
154(Q)	-552	-1780	447	1123	-2047	-1104	22	-1774	497	-1764	-1012	208	-1382	2726	153	-415	-517	-1434	-1946	-1334	154
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
155(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698	-1261	155
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
156(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	156
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
157(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	157
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
158(D)	-1130	-2063	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	158	
)	-1130	-2063	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	158	

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
159(A)																				
)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
160(A)																				
)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
161(E)																				
)	704	-2274	1609	-2494	-2517	-929	-118	-2327	-117	-2302	-1553	440	-1360	238	-777	-462	-729	-1891	-2491	-1671
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
162(A)																				
)	2429	-274	-996	-928	-1860	817	-856	-1435	-970	-1770	-1001	-575	-1171	-793	-1166	139	6	-817	-2094	-1749
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
163(A)																				
)	2297	-276	-974	-901	-1869	1169	-940	-1456	-949	-1779	-1003	-559	-1165	-770	-1154	146	12	-829	-2099	-1749
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
164(W)																				
)	599	-435	-2434	-1855	242	-2141	-835	1806	-1510	1433	984	-1586	-2094	-1181	-1459	-1234	-532	589	2085	-142
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
165(R)																				
)	1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	-332	-1225	-1148	-1717	-1746	-1529
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

166(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	166
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
167(F)	-889	-760	-2459	-2062	-2900	-2246	-675	598	-1781	541	608	-1693	-2303	-1420	-1756	-1432	-885	1145	-260	608	167
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
168(D)	-214	-1405	-1586	576	-1945	-895	-13	-1678	183	-1732	-907	283	1136	366	-351	870	-261	-1244	-1978	-1306	168
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
169(E)	-988	-1921	379	-3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	169
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
170(Q)	-552	-1780	447	1123	-2047	-1104	22	-1774	497	-1764	-1012	208	-1382	153	415	-517	-1434	-1946	-1334	-249	170
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
171(E)	-988	-1921	379	-3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	171
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
172(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	-3069	-557	-1898	-1539	172
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
173(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	-3069	-557	-1898	-1539	173

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

174(V)	-177	-366	-1747	-1376	-835	-1408	-1060	842	-1132	-278	103	-1145	-1767	-1025	-1282	-633	1362	2195	-1521	-1119	174
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

175(A)	2031	-283	-945	-876	-1891	1659	-933	-1488	-939	-1802	-1022	-547	-1163	-758	-1150	148	11	-850	-2114	-1762	175
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

176(I)	-932	-615	-3069	-2624	-731	-2846	-2141	2579	-2386	277	371	-2421	-2767	-2189	-2441	-2065	-921	2203	-1937	-1555	176
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

177(A)	2214	-291	-1447	-1169	-1154	-884	-959	74	-982	-736	-262	-833	-1414	-858	-1151	-156	-103	1000	-1694	-1298	177
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

178(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	3432	-1225	-1148	-1717	-1746	-1529	178
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

179(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	4149	179
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

180(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	180
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

181(P)	-370	-1016	-600	-377	-1687	-1102	-432	-1453	72	-1566	-902	-444	2899	-166	707	-467	-495	-1110	-1727	-1330	181
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
182(F)	-1055	-829	-2811	-2263	2475	-2617	-1120	681	-1966	1473	2107	-2020	-2452	-1505	-1885	-1736	-975	308	-672	-125	182
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
183(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1985	-499	-2112	-1514	3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327	183
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
184(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	184
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
185(I)	-1143	-842	-3082	-2578	-159	-2929	-1882	2333	-2257	1719	917	-2424	-2743	-1919	-2225	-2119	-1091	1050	-1466	-1201	185
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
186(A)	2106	-237	-1021	-871	-1767	-506	-866	-1350	-841	-1674	-895	-527	-1137	-683	-1055	1584	68	-746	-2025	-1642	186
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
187(L)	-1259	-980	-3063	-2554	2	-2960	-1832	1563	-2204	2258	1077	-2433	-2742	-1834	-2157	-2152	-1198	733	-1358	-1124	187
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
188(M)	-1262	-1030	-2945	-2401	126	-2872	-1670	808	-2031	2152	2757	-2305	-2645	-1652	-1984	-2035	-1186	370	-1224	-1041	188

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
189(V)	-851	-606	-2839	-2414	-730	-2622	-1965	1760	-2148	220	330	-2216	-2651	-1999	-2230	-1847	-862	-1875	-1456	189
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
190(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
191(A)	-1930	-244	-1003	-848	-1775	-507	-852	-1365	-819	-1681	-899	-516	-1135	-664	-1041	1787	68	-757	-2027	-1642
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
192(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1660	-1161	-427	-1678	-5540	-59	-855	-904	-1495	-1728	-1237
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
193(C)	763	-1755	-1469	-973	-510	-1023	-517	135	-756	-355	310	-727	-1369	-559	-904	283	843	1335	-953	-582
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
194(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
195(R)	94	-1197	-868	-342	-1810	-1279	-102	-1370	814	-1456	-793	-379	-1529	246	-2803	-535	-501	-1085	-1661	-1286
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

196(P)	-92	-706	-669	-681	-2057	743	-909	-1842	-843	-2030	-1299	-596	3040	-724	-1088	-252	-384	-1257	-2095	-1813	196
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
197(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	197
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
198(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	2927	-1816	-1362	198
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
199(L)	-1198	-902	-3098	-2586	-74	-2954	-1866	1998	-2259	2016	1009	-2444	-2745	-1887	-2211	-2142	-1140	903	-1411	-1174	199
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
200(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	200
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
201(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1660	-1161	-427	-1678	3540	-59	-855	-904	-1495	-1728	-1237	201
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
202(C)	-608	5196	-2368	-2362	-1635	-1253	-1702	-987	-2034	-1540	-1191	-1745	-1831	-1953	-1922	-934	-927	-770	-1855	-1692	202
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
203(S)	1057	-274	-916	-815	-1832	-508	-865	-1436	-820	-1752	-977	-502	-1145	-674	-1039	2412	41	-810	-2079	-1679	203

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

204(V)	-925	-616	-3000	-2497	-527	-2814	-1921	1840	-2252	949	560	-2316	-2703	-1995	-2284	-1988	-889	2430	-1695	-1364	204
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

205(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	205
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

206(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	206
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

207(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	207
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

208(T)	-323	-1596	1187	1153	-1884	-973	55	-1567	265	-1645	-837	335	-1257	437	-273	-205	1701	-1205	-1920	-1239	208
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

209(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	209
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

210(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	210
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

211(E)	-293	-1582	365	1545	-1897	-1040	140	-1597	1076	-1610	-776	280	-1253	546	110	939	-255	-1221	-1825	-1187	211
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
212(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	212
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
213(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	213
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
214(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	4141	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880	214
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
215(R)	-864	-1241	-1101	-424	-1173	-1559	-60	-804	818	181	-343	-483	-1657	246	2582	-741	-578	-719	-1332	-877	215
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
216(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	216
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
217(F)	-707	-668	-1832	-1360	2343	-1971	1711	-39	-1065	993	361	-1104	-1992	-805	-1140	-1047	-642	-73	294	1256	217
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
218(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	218

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

219(C)	1104	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	219
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

220(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	220
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

221(A)	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	221
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

222(E)	-988	-1921	379	-3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	222
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

223(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	-3069	-557	-1898	-1539	223
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

224(I)	-929	-611	-3073	-2627	-742	-2849	-2145	-2502	-2391	262	361	-2423	-2789	-2195	-2447	-2067	-918	2293	-1944	-1560	224
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

225(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	-220	-1038	-1992	-1530	225
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

226(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	2927	-1816	-1362	226
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
227(Y)	-1667	-1473	-2001	-1984	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	4149	227
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
228(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	228
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
229(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	229
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
230(E)	-717	-2337	2049	2259	-2571	-918	-117	-2393	-138	-2352	-1602	461	-1357	240	-821	-464	-744	-1944	-2542	-1698	230
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
231(K)	397	-1331	0	518	-1619	-1070	250	-1306	1059	-1341	-481	786	38	674	793	224	-41	-946	-1579	-969	231
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
232(V)	-700	-665	-2337	-2135	-873	-1865	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	2927	-1816	-1362	232
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
233(F)	-1602	-1340	-2512	-2455	3740	-2315	-379	-478	-2225	-175	-190	-1874	-2503	-1781	-2079	-1892	-1647	-683	210	1257	233
)																					

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

234(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	234
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

235(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	235
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

236(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	236
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

237(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	237
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

238(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	238
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

239(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	239
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

240(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	-3760	-1471	-1571	-1129	-1216	-1780	-2050	-2023	240
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

241(W)	-1926	-1577	-2416	-2370	192	-2070	-795	-1602	-1882	-1347	-1210	-2018	-2383	-1854	-1777	-2150	-1992	-1643	5596	560	241
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
242(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	2786	-220	-1038	-1992	-1530	242
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
243(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	3103	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528	243
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
244(G)	1814	-291	-924	-870	-1911	-1934	-940	-1512	-947	-1824	-1045	-544	-1165	-764	-1158	144	3	-867	-2131	-1779	244
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
245(F)	-1602	-1340	-2512	-2455	3740	-2315	-379	-478	-2225	-175	-190	-1874	-2503	-1781	-2079	-1892	-1647	-683	210	1257	245
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
246(L)	-1359	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2188	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	246
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
247(A)	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	-247	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
248(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	2786	-220	-1038	-1992	-1530	248

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

249(C)	1738	3050	-1597	-1286	-1304	-553	-920	-809	-1074	-1196	-460	-726	-1161	-870	-1191	1014	128	-366	-1662	-1327	249
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

250(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	4149	250
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

251(A)	2806	444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-859	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	251
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

252(S)	116	498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	2786	-220	-1038	-1992	-1530	252
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

253(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	-3432	-1225	-1148	-1717	-1746	-1529	253
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

254(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	254
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

255(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	255
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

256(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	3103	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528	256
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

257(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	4111	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880	257
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

258(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	3452	-1225	-1148	-1717	-1746	-1529	258
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

259(F)	-1868	-1403	-2783	-2661	3327	-2745	337	-941	-2325	-619	-501	-1662	-2699	-1613	-2087	-1932	-1788	-1044	1008	2959	259
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

260(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	260
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

261(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	2786	-220	-1038	-1992	-1530	261
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

262(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	262
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

263(S)	1057	-279	-958	-668	-1633	-564	-673	-1214	-584	-1503	-711	-437	-1133	-450	-856	1823	1396	-673	-1872	-1473	263
)																					

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
264(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
265(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	-2786	-220	-1038	-1992	-1530
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
266(E)	-988	-1921	379	-3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
267(A)	-1795	-308	-1596	-1269	-985	-1098	-1001	487	-1073	-504	-67	-970	-1559	-938	-1234	-348	-181	1695	-1593	-1199
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
268(M)	-485	-517	-1471	-910	-110	-1822	-557	403	-543	1390	-2375	-948	-1827	805	-720	-871	-418	228	-912	-567
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
269(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	-4121	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
270(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

271(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	4149	271
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
272(A)	1700	-251	-1028	-731	-1556	-572	-693	-1113	-631	-1425	-647	-472	-1141	-490	-887	934	1645	-603	-1818	-1429	272
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
273(E)	-988	-1921	379	-3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	273
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
274(G)	307	-371	-641	-650	-1997	-2231	-857	-1752	-837	-1971	-1163	-431	-1164	-654	-1088	1356	-32	-1032	-2185	-1778	274
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
275(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	-3103	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528	275
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
276(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	2786	-220	-1038	-1992	-1530	276
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
277(M)	-965	-982	-2125	-1844	-276	-2022	-1302	373	-1390	610	-4141	-1701	-2229	-1356	-1425	-1444	-1028	126	-1279	-880	277
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
278(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	278

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
279(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
280(L)	-1369	-1189	-2542	-2295	-129	-2453	-1603	488	-1867	742	-2188	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	280
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
281(E)	-988	-1921	379	-3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
282(A)	-244	-1004	-849	-1775	-507	-853	-1364	-820	-1681	-899	-516	-1135	-665	-1041	1773	68	-756	-2027	-1641	282
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
283(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	-1225	-1148	-1717	-1746	-1529	283
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
284(C)	-608	-2368	-2362	-1635	-1253	-1702	-987	-2034	-1540	-1191	-1745	-1831	-1953	-1922	-934	-927	-770	-1855	-1692	284
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
285(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	-2040	-329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698	-1261	285
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

286(Y)	-1934	-1442	-2813	-2708	2952	-2777	363	-1027	-2370	-696	-580	-1669	-2726	-1633	-2118	-1965	-1850	-1121	1040	3463	286
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
287(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	-3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698	-1261	287
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
288(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	288
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
289(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	-3103	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528	289
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
290(A)	-276	-974	-901	-1869	1169	-940	-1456	-949	-1779	-1003	-559	-1165	-770	-1154	146	12	-829	-2099	-1749	290	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
291(A)	-237	-1021	-871	-1767	-506	-866	-1350	-841	-1674	-895	-527	-1137	-683	-1055	1564	68	-746	-2025	-1642	291	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
292(G)	-758	-1103	-1313	-1487	-2460	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	292	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
293(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	-2927	-1816	-1362	293

-	-149	-500	233	43	-381	399	106	-626	210	-486	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
294(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1660	-1161	-427	-1678	3540	-59	-855	-904	-1495	-1728	-1237
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
295(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
296(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
297(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1660	-1161	-427	-1678	3540	-59	-855	-904	-1495	-1728	-1237
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
298(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	-3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
299(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
300(S)	1375	-276	-871	-686	-1859	1354	-765	-1534	-710	-1758	-930	-428	-1109	-536	-985	86	-862	-2062	-1669	300
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

301(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	-2927	-1816	-1362	301
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
302(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	-2786	-220	-1038	-1992	-1530	302
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
303(C)	-608	-5196	-2368	-2362	-1635	-1253	-1702	-987	-2034	-1540	-1191	-1745	-1831	-1953	-1922	-934	-927	-770	-1855	-1692	303
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
304(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	-3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698	-1261	304
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
305(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	305
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
306(M)	-837	-618	-2708	-2158	-178	-2529	-1480	1612	-1882	830	-3103	-1991	-2434	-1567	-1889	-1657	-786	1490	-1279	-1018	306
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
307(P)	180	-461	-726	-522	-1558	-715	-605	-1079	-438	-1388	-684	-414	-2063	-378	-702	3	1812	-655	-1805	-1384	307
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
308(G)	293	-379	-630	-667	-2009	-2397	-884	-1772	-875	-1997	-1196	-444	-1174	-691	-1115	1071	-51	-1049	-2198	-1793	308

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
309(A)		-226	-1055	-939	-1760	-504	-915	-1313	-910	-1669	-905	-561	-1146	-752	-1102	1042	58	-721	-2034	-1657
)	-2390																			309
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
310(V)		-836	-639	-2662	-2225	-512	-2495	-1702	1367	852	487	-2046	-2529	-1758	-1977	-1708	-841	-2598	-1613	-1195
)																				310
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
311(P)		2	-489	-1031	-785	-1108	-956	-714	-305	-612	-789	-314	-653	-567	-828	-238	-203	890	-1569	-1119
)																				311
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
312(S)		311	-370	-673	-637	-1958	759	-820	-1708	-773	-1924	-1114	-425	-1160	-607	-1031	-2407	-18	-1005	-2145
)																				312
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
313(G)		-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198
)																				313
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
314(I)		-1016	-805	-2637	-2367	-591	-2470	-1853	-3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1698
)																				314
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
315(R)		-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	-1225	-1148	-1717	-1746	-1529
)																				315
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

316(A)	-924	62	917	-1556	-884	-105	-1178	129	-1361	-576	63	-1226	230	-299	637	-121	-810	-1700	-1148	316
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
317(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	-1816	-1362	317
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
318(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
319(G)	1867	-289	-929	-871	-1906	-1873	-938	-1506	-944	-1818	-1039	-545	-1165	-761	-1155	146	5	-862	-2126	-1774
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
320(E)	-988	-1921	379	-3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
321(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	-3456	-1574	-435	-810	-661	-818	-1580	-1900	-1327
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
322(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
323(I)	-1123	-822	-3067	-2569	-197	-2917	-1887	2447	-2248	1582	875	-2413	-2743	-1929	-2225	-2110	-1075	1105	-1492	-1210
																				323

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

324(C)	1325	3551	-1712	-1385	-1146	-650	-942	-408	-1136	-968	-296	-812	-1233	-935	-1223	78	996	-79	-1561	-1227	324
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

325(M)	79	-386	-845	-374	-744	-1030	-267	-221	-185	-543	2172	-382	-1318	-104	-470	1220	702	-27	-1118	-688	325
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

326(M)	433	-323	-1005	-574	-677	-1039	-385	-108	-368	-444	2884	-511	-1361	-278	-604	837	-22	63	-1103	-689	326
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

327(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	327
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

328(D)	-1130	-2063	3241	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	328
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

329(L)	-1060	-771	-3035	-2500	-172	-2852	-1816	1635	-2228	1939	929	-2342	-2674	-1854	-2200	-2012	-1004	1515	-1444	-1221	329
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

330(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	330
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

331(C)	-206	3367	-2319	-1919	-689	-1494	-1270	951	-1603	-153	201	-1455	-1861	-1411	-1625	-746	-346	2000	-1422	-1027	331
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
332(A)	2806	444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	332
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
333(S)	116	498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	2786	-220	-1038	-1992	-1530	333
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
334(A)	2078	-244	-950	-773	-1815	720	-810	-1474	-782	-1719	-902	-471	-1112	-603	-1033	1059	91	-820	-2040	-1661	334
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
335(N)	73	2062	-715	-459	-1269	-837	-491	-919	-332	-1269	-568	2714	-1301	-294	-604	-98	-125	-565	-1556	-1049	335
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
336(D)	-1130	-2063	3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	336
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
337(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1660	-1161	-427	-1678	3540	-59	-855	-904	-1495	-1728	-1237	337
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
338(T)	1532	-257	-1123	-898	-1523	-620	-822	-803	-773	-1312	-629	-583	-1207	-654	-978	96	2243	-396	-1854	-1476	338

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

339(F)	-1602	-1340	-2512	-2455	-3740	-2315	-379	-478	-2225	-175	-190	-1874	-2503	-1781	-2079	-1892	-1647	-683	210	1257	339
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

340(S)	337	-317	-860	-701	-1734	-558	-753	-1328	-649	-1636	-869	-457	-1159	-543	-888	1114	-760	-1977	-1560	340
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

341(H)	-1192	-1547	-812	-781	-520	-1557	-456	-1881	-457	-1789	-1357	-866	-1913	-669	-600	-1231	-1258	-1678	-918	-90	341
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

342(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	-220	-1038	-1992	-1530	342
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

343(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	343
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

344(M)	-962	-721	-2832	-2307	-165	-2664	-1626	2366	-1987	903	-2608	-2145	-2549	-1688	-1979	-1822	-914	1020	-1353	-1088	344
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

345(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	-3432	-1225	-1148	-1717	-1746	-1529	345
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

346(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	3432	-1225	-1148	-1717	-1746	-1529	346
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

347(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539	347
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

348(A)	-978	151	960	-1634	273	-124	-1266	89	-1438	-652	85	-1234	211	-352	-94	-159	-886	-1767	-1206	348	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

349(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	3432	-1225	-1148	-1717	-1746	-1529	349
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

350(M)	-441	-455	-1810	-1252	-103	-1867	-749	679	-965	1297	2343	-1183	-1898	-782	-1087	-944	877	573	-915	-594	350
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

351(L)	-1259	-980	-3063	-2554	2	-2960	-1832	1563	-2204	1077	-2433	-2742	-1834	-2157	-2152	-1198	733	-1358	-1124	351	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

352(M)	-73	-469	-1004	-675	-744	1014	-512	-220	-464	-456	3139	-614	-1458	-414	-675	-331	-188	-84	-1209	-778	352
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

353(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1660	-1161	-427	-1678	3540	-59	-855	-904	-1495	-1728	-1237	353
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-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
354(M)																				
)	-997	-819	-2602	-2110	2400	-2453	-884	573	-1803	986	2860	-1838	-2371	-1404	-1756	-1593	-939	253	-467	230
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
355(L)																				
)	-1198	-902	-3098	-2586	-74	-2954	-1866	1998	-2259	2016	1009	-2444	-2745	-1887	-2211	-2142	-1140	903	-1411	-1174
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
356(P)																				
)	1545	-393	-808	-668	-1689	-637	-750	-1218	-653	-1539	-824	-478	2324	-545	-899	53	-48	-726	-1936	-1539
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
357(G)																				
)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
358(T)																				
)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3069	-557	-1898	-1539
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
359(D)																				
)	-1130	-2063	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	359
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
360(F)																				
)	-1902	-1421	-2805	-2692	3214	-2763	351	-984	-2354	-657	-540	-1868	-2714	-1627	-2106	-1950	-1820	-1082	1026	3156
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

361(I)	-1076	-780	-3002	-2529	-309	-2874	-1898	269	-2198	1168	740	-2371	-2742	-1949	-2205	-2081	-1040	1240	-1569	-1227	361
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
362(F)	-151	-497	-1152	-820	2062	-1206	-284	-329	-685	-526	-9	-681	-1538	-520	-889	1555	-260	-181	-431	409	362
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
363(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	2786	-220	-1038	-1992	-1530	363
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
364(G)	-758	-1103	-1313	-1487	-2460	3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	364
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
365(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	2149	365
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
366(S)	1123	-271	-926	-817	-1828	-508	-863	-1430	-818	-1746	-969	-502	-1143	-671	-1038	2382	45	-805	-2074	-1676	366
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
367(A)	2139	-236	-1024	-877	-1766	-505	-870	-1347	-846	-1673	-895	-530	-1137	-689	-1059	1515	68	-744	-2025	-1643	367
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
368(V)	-151	-569	-631	524	-788	-1293	-269	234	-134	-481	122	-371	-1478	-70	-451	-389	637	1708	-1201	-747	368

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
369(P)	-478	-1420	327	1138	-1929	-1032	-248	-1622	-3	-1747	-1039	36	2562	70	-402	-436	-543	-1278	-1965	-1426
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
370(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
371(Y)	-806	-794	-1826	-1397	917	-2006	-90	-29	-1025	35	1798	-1154	-2057	-849	-1103	-1127	-760	-95	211	3271
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
372(D)	-1130	-2063	344	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
373(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
374(M)	-204	-445	-1398	-849	-453	-1403	-637	369	-636	129	3007	-865	-1648	-574	-809	-563	1278	377	-1116	-731
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
375(F)	-1602	-1340	-2512	-2455	3740	-2315	-379	-478	-2225	-175	-190	-1874	-2503	-1781	-2079	-1892	-1647	-683	210	1257
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

[illegible]

-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
384(D)																				
)	-1130	-2063	243	-2502	-1157	-662	-2514	-843	-2354	-657	-540	-1668	-2714	-1627	-2106	-1950	-1820	-1082	1026	3156
-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
385(F)																				
)	-1902	-1421	-2805	-2692	-3214	-2763	351	-984	-2354	-657	-540	-1668	-2714	-1627	-2106	-1950	-1820	-1082	1026	3156
-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
386(D)																				
)	-1130	-2063	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	386
-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
387(D)																				
)	-1130	-2063	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	387
-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
388(Y)																				
)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	388
-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
389(N)																				
)	-199	-701	-549	-93	-228	-1316	21	533	64	-536	109	-1814	-1432	121	-268	-348	-154	-118	-681	1083
-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
390(V)																				
)	-596	-427	-2444	-1942	-648	-2270	-1439	1955	-1702	78	364	-1768	-2320	-1523	-1801	-1418	532	-1522	-1137	390
-	-149	-500	233	43	-381	399	106	-526	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

391(M)	-1151	-859	-3123	-2554	25	-2901	-1760	1894	-2267	1875	-2113	-2393	-2661	-1798	-2177	-2046	-1074	735	-1288	-1136	391
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
392(Q)	-513	-1819	522	1560	-2087	-1069	43	-1821	462	-1801	-1025	265	-1348	2403	72	-362	-480	-1458	-1986	-1342	392
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
393(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	3432	-1225	-1148	-1717	-1746	-1529	393
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
394(D)	-1130	-2063	344	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	394
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
395(L)	-1306	-1067	-2981	-2443	125	-2920	-1717	814	-2052	2337	2190	-2353	-2682	-1686	-2014	-2092	-1231	368	-1248	-1063	395
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
396(M)	872	-604	-643	-123	-730	-1240	-67	-190	798	-479	2200	-252	-1378	160	-90	-281	-76	-42	-1061	-617	396
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
397(V)	-916	-603	-3047	-2601	-754	-2826	-2126	2255	-2365	237	345	-2398	-2775	-2177	-2427	-2043	-906	2503	-1944	-1553	397
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
398(D)	-673	-2181	2496	794	-2461	-904	-143	-2337	-175	-2319	-1575	1933	-1355	203	-844	-448	-722	-1882	-2482	-1647	398

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

399(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	399
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

400(G)	-758	-1103	-1313	-1487	-2460	-3331	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	400
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

401(L)	-1198	-902	-3098	-2586	-74	-2954	-1866	1998	-2259	-2016	1009	-2444	-2745	-1887	-2211	-2142	-1140	903	-1411	-1174	401
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

402(R)	-345	-1126	-591	42	-1183	-1345	1446	-901	819	-996	1243	-126	-1412	479	-1981	-372	-253	-690	-1261	-760	402
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

403(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	-3760	-1471	-1571	-1129	-1216	-1780	-2050	-2023	403
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

404(V)	-886	-604	-2944	-2505	-743	-2731	-2045	1944	-2256	229	340	-2308	-2716	-2086	-2330	-1950	-884	-2675	-1910	-1506	404
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

405(T)	-79	-1241	710	507	-1575	-1016	183	-1259	542	-1330	-485	230	-1179	590	737	676	-1004	-901	-1599	-987	405
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

406(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	406
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
407(E)	39	-1704	551	2053	-1995	-1015	71	-1696	401	-1718	-915	313	-1287	1274	-58	-256	-368	-1326	-1952	-1286	407
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
408(E)	-414	-1855	1337	2003	-2133	-952	45	-1867	223	-1879	-1060	402	-1266	436	-354	-246	592	-1459	-2110	-1370	408
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
409(V)	-82	-237	-1362	-829	-406	-1408	-463	1109	-622	-139	448	-766	-1572	-466	-821	288	937	1440	-906	-511	409
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
410(I)	-1016	-805	-2637	-2367	-591	-2470	-1853	3039	-2040	329	346	-2180	-2589	-1943	-2070	-1879	-1051	1184	-1898	-1261	410
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
411(K)	1191	-905	-299	140	-1432	-1011	-7	-980	1432	-1186	-412	-29	-1257	344	101	-104	626	-667	-1531	-1026	411
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
412(I)	442	-488	-2154	-1781	-680	-1947	-1383	2623	-1524	86	307	-1582	-2159	-1402	-1631	-1169	-575	1239	-1576	-1178	412
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
413(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	3432	-1225	-1148	-1717	-1746	-1529	413

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
414(N)	-406	-1604	364	514	-1650	-1088	1550	-1590	512	-1604	-816	2266	-1324	905	133	-295	-367	-1249	-1692	-1010
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
415(K)	-1028	-1686	-800	-442	-2171	-1521	-263	-1873	3103	-1859	-1251	-547	-1750	74	599	-1004	-972	-1620	-1812	-1528
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
416(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
417(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
418(R)	-426	-1022	-965	-360	-1251	-1374	-109	-634	663	-894	-340	-418	-1549	198	2485	-538	-408	540	-1406	-969
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
419(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
420(I)	-1039	-725	-3101	-2580	-296	-2896	-1918	2322	-2317	1504	807	-2405	-2727	-1971	-2296	-2068	-990	1488	-1558	-1305
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

421(Q)	-862	-1548	-321	-247	-1672	-1336	-419	-1721	130	-1680	-1161	-427	-1678	-3540	-59	-855	-904	-1495	-1728	-1237	421
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
422(A)	-2439	-274	-998	-931	-1859	786	-957	-1433	-972	-1769	-1001	-577	-1172	-795	-1167	139	6	-816	-2094	-1749	422
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
423(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	-2927	-1816	-1362	423
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
424(F)	-1602	-1340	-2512	-2455	-3740	-2315	-379	-478	-2225	-175	-190	-1874	-2503	-1781	-2079	-1892	-1647	-683	210	1257	424
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
425(E)	394	-1554	893	-1329	-1847	-1017	212	-1570	575	-1566	-695	332	-1188	639	992	-66	-138	-1170	-1778	-1108	425
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
426(E)	-547	-2038	1492	-1544	-2334	1402	-46	-2115	24	-2100	-1309	431	-1308	325	-605	-346	-562	-1682	-2320	-1536	426
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
427(L)	-1304	-1065	-2981	-2443	126	-2919	-1716	814	-2063	-2331	2215	-2353	-2680	-1685	-2014	-2091	-1230	368	-1247	-1063	427
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
428(G)	-330	-1125	-91	-18	-1933	-2258	-329	-1639	1029	-1762	-1028	-173	-1409	-22	-158	-369	-453	-1229	-1912	-1465	428

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

429(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	-2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	429
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

430(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	-3760	-1471	-1571	-1129	-1216	-1780	-2050	-2023	430
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

431(Q)	337	-1173	-102	413	-1398	-1116	203	-1043	1032	-735	-333	153	727	-1159	238	-88	-61	-748	-1447	-885	431
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

432(I)	-938	-664	-2954	-2530	-626	-2743	-2041	-2838	-2253	397	449	-2334	-2724	-2070	-2306	-1976	-939	1704	-1845	-1460	432
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

433(T)	737	-257	-1006	-798	-1590	-576	-766	-1064	-703	-1448	-698	-511	-1166	-583	-923	595	-2548	-574	-1871	-1487	433
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

434(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	434
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

435(E)	-988	-1921	379	-3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	435
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

436(E)	-988	-1921	379	3052	-2309	-1196	-495	-2108	-350	-2194	-1612	-103	-1621	-210	-770	-862	-1042	-1799	-2207	-1742	436
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
437(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	-2927	-1816	-1362	437
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
438(E)	-462	-1877	1098	2281	-2164	-948	4	-1884	157	-1917	-1119	395	-1290	383	-418	-292	231	-1485	-2158	-1417	438
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
439(A)	-2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	439
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
440(A)	-2214	-291	-1447	-1169	-1154	-884	-959	74	-982	-736	-262	-833	-1414	-858	-1151	-156	-103	1000	-1694	-1298	440
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
441(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	-3069	-557	-1898	-1539	441
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
442(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	-2149	442
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
443(A)	-2125	-240	-956	-787	-1817	725	-821	-1474	-799	-1722	-906	-477	-1113	-618	-1045	968	90	-818	-2044	-1667	443

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-284	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
444(H)	-241	-1271	205	405	-1420	-1027	-1354	381	-1435	-651	1012	-1286	387	-16	526	-247	-1015	-1560	-893	444
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-284	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
445(G)	115	-698	-127	.9	-1770	-1729	-357	-1446	-174	-1623	822	1088	-1193	-53	-556	21	909	-938	-1917	-1403
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-284	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
446(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	-2786	-220	-1038	-1992	-1530
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-284	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
447(K)	-546	-1647	-8	263	-2054	-1224	81	-1743	-2289	-1711	-938	1349	-1428	473	604	-449	-488	-1398	-1805	-1294
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-284	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
448(D)	-824	-2383	-2964	1142	-2628	-923	-210	-2461	-314	-2455	-1761	430	-1405	123	-1021	-562	-876	-2027	-2629	-1790
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-284	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
449(M)	-764	-700	-2351	-1867	-159	-2218	-1306	906	-1516	830	-3512	-1724	-2266	-1323	-1574	-1392	-764	1232	-1247	-932
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-284	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
450(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	-3760	-1471	-1571	-1129	-1216	-1780	-2050	-2023
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-284	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

451(E)	99	-1520	298	1621	-1816	-1039	179	-1514	1042	-1533	-686	287	196	594	120	-117	-179	-1138	-1759	-1119	451
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
452(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	3432	-1225	-1148	-1717	-1746	-1629	452
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
453(N)	-607	-1987	1547	723	-2300	-917	-146	-2176	-128	-2194	-1447	2758	-1353	195	-745	-420	-660	-1739	-2343	-1556	453
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
454(M)	-798	-549	-2776	-2235	-304	-2552	-1547	1726	-1970	646	2482	-2039	-2467	-1670	-1974	-1687	-751	2029	-1361	-1070	454
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
455(V)	-700	-665	-2337	-2135	-873	-1965	-1727	1107	-1863	-105	22	-1851	-2286	-1794	-1920	-1368	-823	2927	-1816	-1362	455
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
456(E)	-565	-1912	661	2316	-2164	-1033	-4	-1917	326	-1900	-1139	305	-1356	1326	-107	-396	-548	-1547	-2092	-1413	456
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
457(D)	-1130	-2063	3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	457
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
458(M)	-1168	-891	-3079	-2505	76	-2883	-1714	1503	-2212	1892	2710	-2358	-2638	-1740	-2126	-2022	-1087	602	-1243	-1101	458

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
459(K)	182	-1171	-183	203	-1755	-1060	40	-1376	210	-1461	-666	13	-1300	422	353	563	-217	-1012	-1691	-1179
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
460(A)	1995	-401	-1320	-961	1082	-1166	-569	41	-802	-291	135	-791	-1531	-642	-991	-375	-205	143	-927	-304
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
461(A)	1982	-301	-1528	-1215	-1040	-1011	-971	334	-1023	-585	-132	-907	-1498	-891	-1190	-268	-147	1432	-1620	-1225
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
462(Q)	508	-1830	541	1702	-2097	-1060	45	-1834	449	-1813	-1033	277	-1342	2266	48	-354	-475	-1466	-1998	-1347
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
463(E)	565	-2113	1507	2150	-2387	321	-37	-2180	33	-2146	-1350	447	-1307	338	-604	-350	-576	-1736	-2355	-1553
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
464(M)	-1145	-875	-3042	-2468	69	-2853	-1685	1502	-2175	1789	2881	-2322	-2619	-1717	-2097	-1990	-1065	616	-1235	-1085
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
465(M)	-964	-754	-2746	-2216	-92	-2609	-1540	1901	-1884	984	3216	-2071	-2499	-1589	-1883	-1761	-915	868	-1280	-1023
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

466(N)	-212	-1586	953	652	-1877	-1008	188	-1602	1202	-1601	-736	1422	-1199	610	33	-89	555	-1202	-1814	-1139	466
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

467(R)	-957	-1779	-1134	-338	-2324	-1645	124	-1844	1755	-1699	-992	-386	-1687	527	96	-884	-776	-1586	-1694	-1422	467
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

468(N)	-145	-1016	214	150	-2012	1688	-401	-1836	-268	-1957	-1185	2176	-1294	-105	-680	-181	-340	-1299	-2102	-1535	468
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

469(I)	-462	-597	-1498	-959	-506	-1717	-547	2312	-237	-2	349	-933	-1823	-455	761	-864	-432	572	-1103	-678	469
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

470(T)	49	-672	-270	-138	-1544	-795	-385	-1139	-160	-1431	-698	1092	-1260	-119	-475	-71	2357	-741	-1759	-1248	470
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

471(G)	164	-379	-1029	-881	-1405	-2294	-838	-705	-813	-1237	-627	-637	-1312	-705	-1013	-66	-112	840	-1767	-1349	471
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

472(V)	-973	-676	-3010	-2484	-310	-2805	-1822	1723	-2225	1595	781	-2304	-2660	-1894	-2215	-1965	-925	1980	-1517	-1250	472
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

473(D)	-792	-2412	-2801	1455	-2641	-913	-169	-2475	-253	-2443	-1727	459	-1383	174	-964	-525	-834	-2028	-2631	-1771	473
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-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

474(V)	-924	-606	-3067	-2621	-751	-2843	-2142	2385	-2386	247	351	-2416	-2786	-2193	-2444	-2061	-913	2407	-1948	-1560	474
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

475(V)	-886	-604	-2944	-2505	-743	-2731	-2045	1944	-2256	229	340	-2308	-2716	-2086	-2330	-1950	-884	2675	-1910	-1506	475
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

476(K)	-494	-1536	-395	133	-1991	-6	152	-1602	2266	-1562	-774	-53	-1428	560	1152	-424	-406	-1276	-1682	-1238	476
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

477(A)	-2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	477
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

478(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	478
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

479(Y)	499	-364	-899	-371	787	-1296	-82	7	-211	-277	393	-419	-1419	-73	-488	927	-30	144	-587	1023	479
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

480(K)	-84	-1454	654	613	-1738	-1039	285	-1458	1165	-1450	-558	861	-1146	994	487	248	-23	-503	-1655	-1002	480
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

481(H)	-138	-1185	181	434	-1561	117	2574	-1298	368	-1392	-582	681	-1230	426	-73	519	-152	-942	-1647	-1037	481
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
482(G)	-478	-1458	1461	350	-2273	2341	-418	-2092	-439	-2185	-1451	76	-1399	-120	-981	-425	-627	-1604	-2284	-1711	482
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
483(F)	-1075	-853	-2688	-2263	-2905	-2522	-760	1338	-1863	741	748	-1891	-2464	-1545	-1898	-1690	-1030	450	-271	583	483
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
484(K)	-186	-1612	1105	1050	-1898	-1008	220	-1635	1350	-1612	-730	353	-372	652	63	-56	254	-1218	-1811	-1126	484
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
485(D)	-1130	-2063	3441	243	-2502	-1157	-862	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	485
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
486(V)	-913	-603	-3038	-2592	-754	-2818	-2119	-2212	-2355	235	344	-2390	-2769	-2168	-2418	-2035	-904	-2536	-1941	-1549	486
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
487(A)	-2808	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	487
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
488(Q)	-287	-1670	472	1418	-1956	-1010	152	-1686	494	-1676	-825	781	-1230	1735	6	468	-249	-1287	-1883	-1205	488

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
489(N)	982	-1511	1230	624	-1958	-918	1	-1675	186	-1740	-924	1829	-1246	379	-358	-177	-306	-1265	-1993	-1307
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
490(I)	-844	-558	-2919	-2391	-410	-2672	-1718	-2212	-2137	556	1861	-2182	-2573	-1841	-2137	-1823	-801	2017	-1502	-1199
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
491(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	491
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
492(N)	-436	-1887	1302	728	-2163	-972	57	-1926	969	-1904	-1081	2205	-1277	450	-237	-263	-420	-1510	-2102	-1373
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
493(M)	-1145	-875	-3042	-2468	69	-2853	-1685	1502	-2175	1789	2881	-2322	-2619	-1717	-2097	-1990	-1065	616	-1235	-1085
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
494(Q)	438	-738	-549	-83	-752	-1276	-87	-244	206	754	150	-239	-1416	1559	-92	-333	-154	-140	-1136	-668
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
495(K)	-768	-1791	-831	-81	-2307	-1536	199	-1845	-2218	-1687	-922	-205	-1567	1413	1839	-665	-601	-1541	-1705	-1361
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

496(Q)	607	-1101	-64	144	-1616	-1017	-115	-1247	345	-1377	-685	-39	-1340	2700	48	-248	-291	-931	-1715	-1175	496
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

497(K)	-930	-1812	-1098	-282	-2373	-1635	152	-1883	2344	-1718	-994	-346	-1664	564	2242	-842	-743	-1611	-1705	-1425	497
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

498(V)	-925	-616	-3000	-2497	-527	-2814	-1921	1840	-2252	949	560	-2316	-2703	-1995	-2284	-1988	-889	-2430	-1695	-1364	498
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

499(C)	375	-2958	-1479	-1118	-1141	-638	-785	-629	-894	-1021	-301	-681	-1196	-724	-1042	842	1871	-256	-1509	-1153	499
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

500(G)	-758	-1103	-1313	-1487	-2460	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	-2279	500
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

501(D)	-1130	-2063	-6241	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	501
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

502(Y)	-1894	-1443	-2713	-2588	2338	-2734	353	-1021	-2261	-702	-584	-1632	-2696	-1582	-2053	-1924	-1818	-1110	1018	3836	502
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

503(L)	-1369	-1199	-2542	-2295	-129	-2453	-1603	488	-1867	2607	742	-2168	-2539	-1724	-1835	-1992	-1384	130	-1284	-897	503
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
504(Q)	-528	-1553	-18	249	-1355	-1246	1676	-1537	686	-1512	-787	17	-1438	2674	436	-447	-468	-1245	-1443	-765
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
505(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	3039	-557	-1898	-1539
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
506(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	-2786	-220	-1038	-1992	-1530
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
507(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
508(I)	-938	-642	-3015	-2577	-673	-2799	-2092	-2768	-2321	349	419	-2380	-2757	-2128	-2375	-2024	-932	1888	-1889	-1511
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
509(F)	-1021	-751	-2984	-2430	-1835	-2690	-1365	1831	-2140	1558	1076	-2171	-2517	-1668	-2032	-1822	-947	667	-916	-487
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
510(D)	-228	-933	1576	139	-980	-1247	-106	-126	97	59	-65	-98	-1412	196	-332	-320	-202	651	-1373	-816
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

511(K)	-125	-1470	735	601	-1768	-117	251	-1483	1009	-1480	-601	299	-1169	685	990	468	-71	-1087	-1689	-1041	511
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
512(Q)	-215	-1686	744	1127	-1974	-31	209	-1725	1008	-1685	-799	954	-1185	1224	24	-72	-169	-1293	-1872	-1170	512
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
513(W)	-590	-560	-1824	-1369	2316	64	40	-143	-1101	-278	223	-1055	-1904	-810	-1154	-929	-548	-80	3223	1325	513
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
514(H)	-169	-1619	601	1061	-1918	-1017	1341	-1660	1041	-1619	-725	901	-1171	1254	132	-37	-116	-1232	-1801	-1117	514
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
515(V)	-337	1937	-2456	-2043	-721	-1722	-1414	1163	-1743	-109	212	-1622	-2030	-1552	-1771	-966	-446	2551	-1519	-1120	515
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
516(V)	-571	-393	-2384	-1841	-446	-2249	-1248	1704	-1594	738	539	-1678	-2244	-1375	-1669	-1360	519	2046	-1284	-924	516
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
517(S)	116	-498	-840	-867	-1751	-671	-949	-1625	-916	-1896	-1218	-622	-1304	-824	-1097	2786	-220	-1038	-1992	-1530	517
)	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
518(A)	2806	-444	-1270	-1265	-1732	-744	-1182	-1034	-1212	-1545	-1010	-869	-1380	-1102	-1325	-156	-259	-640	-2039	-1721	518
)																					

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

519(V)	-886	-604	-2944	-2505	-743	-2731	-2045	1944	-2256	229	340	-2308	-2716	-2086	-2330	-1950	-884	-2675	-1910	-1506	519
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

520(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	-3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327	520
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

521(D)	-710	-2209	-2783	792	-2489	-901	-175	-2383	-242	-2372	-1646	1391	-1370	162	-925	-480	-769	-1927	-2525	-1680	521
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

522(I)	926	1569	-2066	-1523	-360	-1740	-859	-1653	-1268	36	522	-1286	-1866	-1039	-1329	-859	-244	-1499	-985	-613	522
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

523(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1985	-499	-2112	-1514	-3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327	523
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

524(D)	-1130	-2063	-3441	243	-2502	-1157	-662	-2514	-843	-2578	-2029	-96	-1658	-421	-1434	-970	-1239	-2138	-2388	-1915	524
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

525(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	-2149	525
)																					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

526(Q)	394	-1108	-203	338	-1300	-1159	184	-918	1042	-1043	968	94	-1255	277	-133	-76	-656	-1380	-845	526
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

527(G)	-758	-1103	-1313	-1487	-2460	-333	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	527
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

528(P)	-934	-1220	-1352	-1451	-2187	-1314	-1456	-2160	-1488	-2268	-1827	-1359	-3760	-1471	-1571	-1129	-1216	-1780	-2050	-2023	528
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

529(G)	1163	-332	-849	-861	-1983	-2434	-981	-1591	-996	-1909	-1142	-553	-1190	-813	-1198	105	-47	-932	-2188	-1842	529
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

530(T)	-52	-555	-1173	-1112	-1571	-872	-1041	-862	-950	-1353	-866	-843	-1455	-937	-1084	-285	-3069	-557	-1898	-1539	530
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

531(G)	-758	-1103	-1313	-1487	-2460	-333	-1562	-2502	-1720	-2644	-2081	-1324	-1775	-1590	-1790	-958	-1093	-1921	-2198	-2279	531
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

532(Y)	-1667	-1473	-2001	-1964	1181	-2210	-25	-1224	-1658	-1018	-871	-1505	-2415	-1423	-1618	-1747	-1692	-1248	501	-4149	532
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

533(R)	-1181	-1614	-1431	-903	-2066	-1632	-446	-1944	559	-1867	-1326	-899	-1873	-156	-3432	-1225	-1148	-1717	-1746	-1529	533
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-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

534(L)	-1227	-936	-3091	-2579	-37	-2962	-1855	1807	-2244	2137	1045	-2445	-2745	-1867	-2194	-2151	-1166	827	-1387	-1156	534
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

535(W)	-110	-1096	-68	835	-1238	-1114	184	-918	540	-1049	-251	146	-1227	1144	118	925	-52	-647	1432	-790	535
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

536(E)	374	-1428	234	1314	-1721	-59	206	-1425	962	-1455	-596	292	-335	625	87	-51	-101	-1049	-1693	-1050	536
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-660	-6045	-1506	-894	-1115	-701	-1378	*	*												

537(D)	-431	-1310	2676	692	-1656	-655	-71	-1520	-129	-1690	-1117	380	-1112	179	-645	-333	-523	-1206	-1668	-1155	537
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-50	-5435	-6477	-894	-1115	-1219	-810	*	*												

538(K)	-15	-765	-73	241	-1227	-873	152	-678	1382	-945	-263	98	-1144	476	501	-77	963	-426	-1324	-852	538
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-50	-5435	-6477	-894	-1115	-451	-1896	*	*												

539(E)	-567	-2146	1487	2236	-2383	-935	-11	-2178	98	-2131	-1330	451	-1305	974	-516	-347	-566	-1739	-2332	-1532	539
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

540(R)	-635	-1728	-475	809	-2174	-1411	191	-1754	1549	-1647	-864	-79	-1485	810	2276	-530	-501	-1435	-1716	-1303	540
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												

541(W)	-1926	-1577	-2416	-2370	192	-2070	-795	-1502	-1882	-1347	-1210	-2018	-2383	-1854	-1777	-2150	-1992	-1643	-5696	560	541
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
542(E)	376	-1973	1778	-1849	-2250	-935	10	-2003	135	-2000	-1190	428	-1283	394	-473	-289	-479	-1580	-2225	-1454	542
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
543(E)	-502	-1664	66	-1892	-2010	-1207	100	-1682	762	-1657	-874	90	-1392	499	1653	-394	-434	-1344	-1798	-1268	543
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
544(I)	-1076	-780	-3002	-2529	-309	-2874	-1898	-2597	-2198	1168	740	-2371	-2742	-1949	-2205	-2081	-1040	1240	-1569	-1227	544
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
545(K)	-636	-1780	35	978	-2175	-1268	89	-1797	-2425	-1748	-984	44	-1457	488	647	-514	-552	-1469	-1853	-1366	545
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
546(N)	-637	-1349	-5	-148	-1773	-1078	-624	-1965	-499	-2112	-1514	-3456	-1574	-435	-810	-661	-818	-1560	-1900	-1327	546
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
547(I)	-985	-659	-3098	-2595	-475	-2895	-1995	-2517	-2348	1018	628	-2411	-2755	-2059	-2358	-2076	-945	1794	-1699	-1395	547
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*												
548(P)	1061	-433	-770	-684	-1731	-657	-791	-1256	-689	-1580	-892	-489	-2706	-594	-921	8	-100	-769	-1876	-1581	548

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
549(W)	-38	-416	-726	-186	608	365	-32	-47	-33	-324	375	490	-1365	81	-348	-269	377	100	1582	549
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
550(A)	-301	-1528	-1215	-1040	-1011	-971	334	-1023	-585	-132	-907	-1498	-891	-1190	-268	-147	1432	-1620	-1225	550
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
551(M)	-956	-675	-3000	-2437	-98	-2720	-1640	1822	-2169	1596	-1921	-2230	-2553	-1752	-2099	-1856	-891	1429	-1284	-1077
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
552(D)	-409	-1868	-1976	711	-2139	-994	95	-1893	949	-1858	-1023	375	-1268	1572	-112	-239	-379	-1478	-2048	-1334
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
553(P)	-934	-1220	-1352	-1451	-2187	-1314	-1458	-2160	-1489	-2268	-1827	-1359	-1760	-1471	-1571	-1129	-1216	-1780	-2050	-2023
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
554(Q)	-349	-1867	1242	1165	-2138	-348	120	-1907	361	-1863	-1004	1155	-1230	-1761	-205	-176	-320	-1469	-2057	-1314
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											
555(E)	-176	-1613	862	1081	-1899	-1001	224	-1641	1004	-1615	-729	978	-1172	658	47	503	231	-1220	-1813	-1122
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-33	-6045	-7087	-894	-1115	-701	-1378	*	*											

[illegible]

TABLE 13

HMIMER2.0 [2.3.2]

NAME beta_exp_seqs

LENG 238

ALPH Amino

RF no

CS no

MAP yes

COM hmmbuild beta_hmm2 beta_exp_seqs.a

COM hmmscalibrate beta_hmm2

NSEQ 8

DATE Fri Mar 30 18:30:25 2007

CKSUM 9853

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NULT -4 -8455

NULE 595 -1558 85 338 -294 453 -1158 197 249 902 -1085 -142 -21 -313 45 531 201 384 -1998 -644

EVD -152.743744 0.132052

HMM	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
	m->m	m->i	m->d	i->m	i->i	d->m	d->d	b->m	m->e											
	-561	*	-1634																	
1(M)	-117	-798	-402	82	-937	-1185	8	-544	307	-732	2122	-103	-1331	1400	-38	722	-88	-348	-1198	-701
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-31	-6123	-7165	-894	-1115	-701	-1378	-561	*											
2(E)	-1082	-2012	315	3129	-2414	-1266	-581	-2231	-449	-2306	-1728	-179	-1697	-299	-873	-951	-1139	-1916	-2295	-1840
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*											
3(I)	-1084	-859	-2738	-2477	-657	-2548	-1955	-3104	-2151	274	286	-2280	-2665	-2051	-2174	-1970	-1120	1153	-1775	-1342
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*											
4(D)	-728	-2252	2483	757	-2533	-947	-190	-2414	-229	-2389	-1646	2098	-1401	156	-906	-498	-777	-1953	-2549	-1708
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*											

5(E)	-1082	-2012	315	~~3129~~	-2414	-1266	-581	-2231	-449	-2306	-1728	-179	-1697	-299	-873	-951	-1139	-1916	-2295	-1840	7
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

6(T)	-222	-1283	7	357	-1665	-1092	81	-1315	1142	-1403	-595	1311	-1290	468	179	-182	~~1459~~	-978	-1664	-1099	8
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

7(L)	-1470	-1280	-2669	-2428	-178	-2553	-1713	430	-2000	~~2666~~	707	-2294	-2628	-1837	-1953	-2111	-1483	53	-1355	-983	9
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

8(L)	-1470	-1280	-2669	-2428	-178	-2553	-1713	430	-2000	~~2666~~	707	-2294	-2628	-1837	-1953	-2111	-1483	53	-1355	-983	10
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

9(R)	-1293	-1707	-1553	-1016	-2181	-1720	-534	-2070	488	-1979	-1441	-1002	-1962	-244	~~3506~~	-1337	-1257	-1841	-1832	-1633	11
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

10(N)	-492	-1682	390	456	-1956	-1087	-26	-1802	395	-1805	-1034	~~2238~~	-1386	2034	28	-379	-488	-1428	-1929	-1277	12
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

11(I)	-1084	-859	-2738	-2477	-657	-2548	-1955	~~3104~~	-2151	274	286	-2280	-2665	-2051	-2174	-1970	-1120	1153	-1775	-1342	13
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

12(I)	-1084	-859	-2738	-2477	-657	-2548	-1955	~~3104~~	-2151	274	286	-2280	-2665	-2051	-2174	-1970	-1120	1153	-1775	-1342	14
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

13(K)	-505	-1727	-252	1422	-2133	-1318	183	-1765	~~1640~~	-1672	-851	28	-1413	612	1584	-390	-399	-1407	-1768	-1274	15
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

14(D)	-658	-2242	~~2252~~	1435	-2514	853	-109	-2321	-87	-2279	-1497	422	-1362	259	-753	-428	-678	-1868	-2487	-1660	16
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-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*											

15(V)	748	-413	-2029	-1720	-969	-1515	-1368	951	-1502	-332	-2	-1391	-1912	-1365	-1614	-776	-423	2546	-1740	-1342	17
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

16(L)	-1296	-988	-3217	-2703	-76	-3057	-1956	1866	-2372	2163	1015	-2560	-2822	-1967	-2303	-2251	-1234	794	-1447	-1233	18
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

17(N)	1158	-703	-271	-42	-1756	-755	-333	-1434	-123	-1598	-780	1499	-1204	-10	-522	1429	-63	-929	-1892	-1374	19
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

18(E)	-1082	-2012	315	3229	-2414	-1266	-581	-2231	-449	-2306	-1728	-179	-1697	-299	-873	-951	-1139	-1916	-2295	-1840	20
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-701	-1378	*	*												

19(M)	-510	-499	-2163	-1628	-454	-1946	-1027	664	-1344	132	3301	-1444	-2063	-1141	-1422	-1064	853	1357	-1169	-825	21
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*												

20(Q)	-595	-1941	-47	1215	-2308	-1349	8	-1994	1563	-1906	-1064	12	-1502	2357	337	-457	-516	-1599	-2025	-1446	22
-	-149	-498	233	43	-381	399	106	-626	210	-466	-720	275	394	46	96	360	118	-369	-294	-249	
-	-587	-1605	-7537	-189	-3027	-701	-1378	*	*												

21(T)	-109	-928	-430	-253	-2011	1064	-575	-1692	-379	-1854	-1040	1320	-1426	-258	-774	-200	2174	-1178	-2146	-1631	24
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*												

22(S)	546	-1052	-378	86	-1686	381	-156	-1358	153	-1477	-642	-105	-1332	1007	-293	5168	1023	-960	-1778	-1219	25
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*												

23(D)	-695	-2280	2543	1073	-2545	-1168	-161	-2333	770	-2265	-1430	221	-1490	1100	-543	-490	-678	-1880	-2447	-1670	26
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*
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24(K)	-362	-1292	-506	92	-1508	-1378	26	-46	-1229	-447	-128	-1457	402	833	-358	1354	-842	-1539	-1035	27
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249	
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*											

25(P)	277	-1467	-164	225	-1828	-1260	-33	-1485	934	-1547	-732	-28	1055	98	-326	-342	-1148	-1799	-1237	28
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249	
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*											

26(I)	-636	-547	-2348	-1836	-669	-2184	-1282	-2090	-1586	-84	272	-1663	1335	-1395	-1679	-1311	1784	-1415	-1037	29
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249	
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*											

27(S)	-24	-544	-1523	-1279	-1545	-999	-1116	-753	-1130	-1366	-748	-952	-1562	-1005	-1316	-2493	-298	1210	-1962	-1530	30
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249		
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*												

28(F)	-2311	-1922	-3157	-3219	-24089	-2828	-763	-1194	-2995	-797	-853	-2459	-3032	-2434	-2736	-2546	-2370	-1425	-113	961	31
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249		
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*												

29(I)	-205	-1415	-93	368	-1801	194	53	-1500	1106	-1542	-677	847	-1311	472	-33	450	11223	-1112	-1785	-1163	32
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249		
-	-24	-6495	-7537	-894	-1115	-701	-1378	*	*												

30(M)	1277	-412	-1511	-962	-449	92	-534	78	-746	841	51781	-899	-1714	-574	-925	-643	-242	174	-924	-542	33
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249		
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

31(Q)	-302	-1783	866	1044	-2094	-1206	84	-1848	842	-1794	-879	744	-16	1626	-45	369	-247	-1401	-1967	-1274	34
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249		
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

32(A)	1179	-775	528	-218	-885	-1427	-232	-328	-104	479	11	-384	-1539	-5	-477	-420	394	364	-1220	-755	35
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	96	359	117	-369	-294	-249		
-	-199	-6672	-3068	-894	-1115	-701	-1378	*	*												

33(K)	737	-1279	-218	331	-1510	-1210	95	-1172	1077	-1272	-440	61	339	495	575	312	-129	-85	-1572	-988	36
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-675	-6497	-1463	-894	-1115	-990	-1010	*	*												
34(S)	1592	-166	-797	-644	-1616	-422	-681	-1189	-623	-1515	-745	-366	-1034	-483	-858	1842	163	-617	-1871	-1468	37
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-37	-5859	-6901	-894	-1115	-1577	-589	*	*												
35(A)	1794	-226	-1352	-1036	-937	-920	-827	406	-846	-478	-30	-769	-1396	-729	-1028	-171	-53	1373	-1511	-1108	38
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-37	-5859	-6901	-894	-1115	-1577	-589	*	*												
36(A)	1875	-154	-826	-672	-1598	-421	-693	-1162	-644	-1496	-730	-380	-1035	-502	-873	1543	167	-597	-1860	-1462	39
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-	-37	-5859	-6901	-894	-1115	-1577	-589	*	*												
37(S)	326	-378	-424	-280	-1605	-535	-464	-1316	-311	-1530	-754	-204	1087	-215	-611	1952	64	-770	-1804	-1337	40
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-37	-5859	-6901	-894	-1115	-1577	-589	*	*												
38(A)	1607	-764	-122	105	-1345	-864	-110	-927	240	-1134	-440	-28	-1219	1445	-70	-82	-102	-620	-1536	-1024	41
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-37	-5859	-6901	-894	-1115	-1577	-589	*	*												
39(A)	1803	-165	-972	-743	-1388	-531	-683	-645	-621	-1166	-492	-458	-1107	-509	-835	189	1642	-256	-1723	-1338	42
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-262	-5859	-2747	-894	-1115	-1577	-589	*	*												
40(T)	355	-282	-441	-217	-1238	-551	-308	-723	-120	-1049	-364	-157	1124	-76	-394	184	1668	-353	-1502	-1065	43
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-44	-5640	-6682	-894	-1115	-1694	-534	*	*												
41(V)	1381	-162	-1227	-907	-788	-898	-715	613	-712	-293	125	-683	-1345	-611	-902	-143	7	1512	-1390	-978	44
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-44	-5640	-6682	-894	-1115	-1694	-534	*	*												

42(I)	1348	-93	-776	-557	-1228	-464	-527	-425	-433	-977	-333	-323	-1020	-345	-654	258	732	-77	-1578	-1179	-249	45
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249		
-	-44	-5640	-6682	-894	-1115	-1250	-787	*	*													

43(A)	1932	-257	-1412	-1102	-1001	-936	-884	327	-912	-552	-96	-813	-1424	-790	-1089	-191	-85	1321	-1569	-1170	46
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-35	-5944	-6986	-894	-1115	-1199	-825	*	*												

44(A)	2374	-274	-1256	-1069	-1658	-626	-968	-939	-950	-1454	-768	-679	-1243	-815	-1130	75	1222	-496	-1989	-1628	47
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-1391	-693	*	*												

45(P)	-1069	-1335	-1496	-1611	-2330	-1424	-1603	-2332	-1655	-2427	-1993	-1508	3827	-1636	-1724	-1268	-1357	-1941	-2164	-2168	48
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-1391	-693	*	*												

46(V)	-182	-442	-1500	-1190	-857	-1313	-928	462	-980	-418	3	-1007	702	-888	-1142	-563	-322	241	-1454	-1039	49
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-1391	-693	*	*												

47(A)	1619	-670	-305	-86	-1730	-748	-364	-1400	-169	-1579	-768	1506	-1208	-53	-557	903	-62	-901	-1883	-1375	50
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-1391	-693	*	*												

48(G)	-883	-1213	-1453	-1640	-2599	3389	-1702	-2667	-1883	-2795	-2238	-1465	-1887	-1747	-1938	-1085	-1224	-2070	-2309	-2422	51
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-302	-6123	-2519	-894	-1115	-1391	-693	*	*												

49(D)	1124	-1254	2055	577	-1863	-791	-92	-1454	1	-1671	-928	298	-1204	245	-533	-149	-294	-1070	-1981	-1326	52
-	-149	-500	233	43	-381	399	106	-626	211	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-1023	-2195	-1788	-353	-2203	-1133	-879	*	*												

50(D)	-523	-2067	2082	1864	-2301	-772	50	-2094	60	-2087	-1336	595	-1196	407	-594	-290	-546	-1670	-2291	-1479	54
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												

51(G)	-313	-704	-806	-934	-1944	3026	-1044	-1895	-1126	-2082	-1505	-815	-1368	-1020	-1245	-501	-624	-1373	-1779	-1747	55
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-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												
52(P)	1565	-250	-551	-379	-1407	-503	-474	-908	-350	-1239	-536	-247	1592	-257	-615	205	126	470	-1666	-1248	56
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												
53(E)	1104	-1069	435	1528	-1553	-836	16	-1056	263	-1313	-590	250	-1178	352	-163	-109	-176	-750	-1687	-1104	57
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												
54(P)	-451	-806	-833	-877	-1665	-916	-922	-1540	-883	-1690	-1226	-823	3400	-878	-1016	-633	-706	-1199	-1631	-1492	58
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												
55(E)	-667	-1586	578	2707	-1926	-955	-206	-1660	-16	-1789	-1202	141	-1361	84	-417	-562	-713	-1376	-1884	-1396	59
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												
56(K)	-702	-1385	-456	-137	-1799	-1249	-32	-1476	2770	-1521	-915	-261	-1487	298	734	-689	-663	-1234	-1555	-1219	60
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												
57(K)	-242	-1146	-42	266	-1604	-1019	110	-1246	1719	-1332	-591	71	1256	469	482	-230	-239	-934	-1543	-1063	61
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												
58(V)	-692	-560	-2296	-1824	-148	-2252	-1309	1284	-1503	1481	845	-1707	-2262	-1329	-1585	-1428	-681	1916	-1248	-872	62
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-41	-5738	-6781	-894	-1115	-1644	-556	*	*												
59(D)	-368	-1609	2275	599	-2245	503	-149	-2013	-61	-2043	-1235	290	-1304	215	-647	738	-448	-1529	-2272	-1549	63
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-1391	-693	*	*												
60(W)	-1818	-1380	-2886	-2725	2827	-2739	204	-861	-2341	-518	-402	-1751	-2707	-1673	-2100	-1954	-1745	-988	4383	2002	64
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-31	-6123	-7165	-894	-1115	-1391	-693	*	*
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61(F)	-1369	-1089	-2974	-2547	~~2668~~	-2815	-904	479	-2229	1773	892	-2159	-2660	-1714	-2105	-1996	-1299	90	-319	513	65
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-1391	-693	*	*												

62(Q)	-318	-1420	-175	306	-1747	-1213	155	-1383	1463	-1416	-607	66	~~1585~~	-1336	492	-256	1223	-1062	-1632	-1103	66
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-31	-6123	-7165	-894	-1115	-393	-2068	*	*												

63(H)	-965	-2315	304	2557	-2345	~~2332~~	-1402	-2376	-96	-2333	-1572	-69	-1734	-25	-542	-787	-951	-1991	-2354	-1617	67
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

64(V)	-243	-624	-951	-402	-698	164	-280	507	700	-540	158	-512	-1577	-129	-534	129	-199	~~1501~~	-1065	-632	68
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

65(G)	-1103	-2683	1617	1044	-3018	~~22466~~	-544	-2852	-622	-2806	-2065	78	-1745	-197	-1335	-856	-1156	-2377	-3010	-2151	69
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

66(I)	127	-601	-1729	700	-741	-2010	-897	~~1823~~	-1032	-346	165	-1212	-2069	-884	-1251	-1065	-513	1823	-1321	-915	70
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

67(A)	~~3260~~	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619	71
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

68(K)	-672	-1700	-767	-106	-2028	-1594	-55	-1633	~~2386~~	-139	-843	-300	-1654	352	1135	118	-565	-1339	-1812	-1353	72
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

69(P)	810	-1146	-439	-69	-1871	-1144	-337	-1545	-33	-1667	-841	-243	~~2101~~	1026	-457	727	-307	-1127	-1984	-1414	73
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

70(G)	-1150	-2176	1681	-110	-3126	~~2890~~	-1035	-3045	-1194	-3069	-2369	-410	-1958	-753	-1812	-1057	-1331	-2472	-3057	-2480	74
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
71(E)	-251	-1222	-293	~~1202~~	-1362	-1299	3	-1014	313	-180	-363	912	-1397	370	-141	-248	901	-750	-1509	844	75
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
72(Q)	-574	-1746	-481	54	-2162	-1456	-57	-1827	650	-1790	-959	-177	-1574	~~2167~~	1643	1133	-504	-1461	-1934	-1408	76
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
73(Q)	181	-1571	-198	359	-1847	-1263	91	-1546	1095	-1559	-684	70	-1354	~~1506~~	888	829	-211	-322	-1779	-1157	77
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
74(D)	-1284	-2925	~~13272~~	428	-3206	-1301	-666	-3170	-865	-3100	-2422	1520	-1826	-343	-1846	-999	-1367	-2663	-3228	-2302	78
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
75(E)	-1877	-2708	-271	~~13539~~	-3235	-1868	-1322	-3210	-1299	-3203	-2676	-849	-2342	-1076	-1736	-1723	-1961	-2857	-2976	-2640	79
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
76(V)	-1294	-1167	-3176	-3053	-1510	-2597	-2599	776	-2808	-665	-568	-2665	-2928	-2721	-2804	-2093	-1429	~~3343~~	-2527	-2099	80
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
77(V)	-1387	-983	-3817	-3421	-1325	-3517	-3036	2126	-3234	-247	-157	-3173	-3403	-3060	-3284	-2792	-1380	~~2968~~	-2682	-2263	81
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
78(I)	-1608	-1307	-3432	-3238	-1179	-3095	-2687	~~3474~~	-2941	-191	-206	-2987	-3216	-2818	-2915	-2635	-1653	832	-2363	-1952	82
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

79(G)	2003	-693	-1730	-1779	-2673	~~2541~~	-1744	-2343	-1885	-2638	-1819	-1201	-1639	-1627	-1995	-291	-469	-1512	-2872	-2608	83
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
80(V)	-1377	-984	-3782	-3387	-1310	-3475	-2994	1952	-3192	-238	-150	-3138	-3378	-3020	-3242	-2751	-1373	~~3030~~	-2656	-2235	84
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
81(G)	2256	-685	-1743	-1778	-2653	~~2305~~	-1732	-2321	-1869	-2616	-1796	-1198	-1633	-1613	-1982	-284	-459	-1496	-2856	-2589	85
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
82(P)	-2089	-2190	-2567	-2787	-3372	-2253	-2663	-3607	-2895	-3590	-3215	-2612	~~4022~~	-2846	-2839	-2316	-2418	-3135	-2975	-3234	86
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
83(A)	~~23260~~	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619	87
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
84(F)	-2605	-2164	-3410	-3518	~~2486~~	-3034	-940	-1504	-3300	-1072	-1142	-2698	-3246	-2697	-2999	-2815	-2670	-1743	-274	806	88
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
85(D)	1368	-1571	~~21780~~	109	-2469	1557	-540	-2201	-430	-2276	-1455	-143	-1580	-184	-971	-465	-630	-1675	-2520	-1865	89
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
86(K)	-543	-1715	-308	1446	-1982	-1429	-43	-1616	~~1943~~	117	-835	-107	-1542	369	238	-460	-465	-1300	-1873	-1318	90
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
87(Y)	768	-433	-1427	-878	-230	-1635	1002	28	-656	-299	359	-841	-1719	-495	-842	-672	-251	1365	-705	~~2062~~	91
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
88(Q)	-713	-1108	-1266	-711	-658	-1906	-585	-189	-306	733	1857	-852	-1945	~~2725~~	-557	-934	-646	-321	-1349	-939	92

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

89(H)	-509	-1727	1105	324	-1970	-1263	2947	-1737	98	-1797	-978	19	-1505	215	-403	-416	1593	-1376	-2025	-1356	93
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

90(H)	257	-1526	-246	303	-1778	-1296	1995	-1458	1182	-1499	-646	24	-1387	1802	104	-236	-241	-136	-1741	-1141	94
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

91(N)	-205	-1061	-522	-287	-2096	-1074	-595	-1786	-360	-1930	-1104	2136	-1508	-259	-766	834	2022	-1276	-2209	-1676	95
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

92(M)	-1753	-1370	-3997	-3423	-278	-3613	-2472	2205	-3134	2048	2292	-3216	-3244	-2474	-2907	-2799	-1664	281	-1734	-1673	96
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

93(V)	-961	-720	-3067	-2568	-1001	-2736	-1942	2099	-2323	-277	18	-2312	-2758	-2110	-2361	-1900	1460	2254	-1899	-1513	97
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

94(G)	-798	-2252	1834	409	-2613	1891	-331	-2390	1009	-2358	-1542	66	-1618	55	-720	-619	-806	-1944	-2552	-1804	98
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

95(M)	-1293	-943	-3609	-3096	-758	-3225	-2328	2635	-2838	287	2654	-2820	-3076	-2478	-2765	-2408	-1249	1673	-1950	-1676	99
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

96(P)	-396	-1347	-270	-70	-2090	-1203	-421	-1805	-70	-1895	-1089	-261	2547	1365	-449	727	-492	-1369	-2141	-1577	100
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

97(H)	-2323	-2419	-1868	-1956	-1368	-2396	5124	-3143	-1645	-2924	-2595	-1989	-2806	-1873	-1712	-2381	-2439	-2923	-1719	-935	101
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

[illegible]

106(A)	3260	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619	110
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

107(G)	-1814	-2025	-2478	-2748	-3577	3650	-2700	-3847	-3052	-3864	-3354	-2492	-2699	-2874	-2984	-2040	-2187	-3148	-3081	-3439	111
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

108(I)	-1408	-993	-3860	-3463	-1315	-3565	-3081	2912	-3283	-218	-139	-3218	-3431	-3098	-3328	-2843	-1397	2338	-2695	-2286	112
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

109(E)	-1877	-2708	-271	3539	-3235	-1868	-1322	-3210	-1299	-3203	-2676	-849	-2342	-1076	-1736	-1723	-1961	-2857	-2976	-2640	113
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

110(E)	-1877	-2708	-271	3539	-3235	-1868	-1322	-3210	-1299	-3203	-2676	-849	-2342	-1076	-1736	-1723	-1961	-2857	-2976	-2640	114
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

111(E)	-1877	-2708	-271	3539	-3235	-1868	-1322	-3210	-1299	-3203	-2676	-849	-2342	-1076	-1736	-1723	-1961	-2857	-2976	-2640	115
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

112(G)	-1814	-2025	-2478	-2748	-3577	3650	-2700	-3847	-3052	-3864	-3354	-2492	-2699	-2874	-2984	-2040	-2187	-3148	-3081	-3439	116
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

113(L)	-1899	-1496	-4056	-3537	-330	-3780	-2667	1776	-3219	2526	815	-3381	-3385	-2604	-3022	-3020	-1820	326	-1861	-1762	117
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

114(K)	-1189	-2157	-978	-437	-2386	-1859	2668	-2247	-2096	-1365	-571	-1954	163	690	-1086	-1045	-1950	-1998	-1554	118
-	-149	-500	233	43	-381	399	106	-626	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											
115(A)	2868	-600	-1841	-1808	-2491	-872	-1666	-2124	-1780	-1635	-1169	-1579	-1551	-1883	1107	-372	-1350	-2748	-2442	119
-	-149	-500	233	43	-381	399	106	-626	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											
116(R)	-2176	-2415	-2504	-1923	-3054	-2403	-1269	-3053	-167	-2856	-2350	-1844	-2662	-997	-2228	-2131	-2807	-2501	-2452	120
-	-149	-500	233	43	-381	399	106	-626	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											
117(V)	-1376	-984	-3781	-3386	-1309	-3474	-2993	1947	-3191	-238	-150	-3136	-3377	-3019	-3241	-2749	-1373	-2655	-2234	121
-	-149	-500	233	43	-381	399	106	-626	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											
118(V)	-1387	-983	-3817	-3421	-1325	-3517	-3036	2126	-3234	-247	-157	-3173	-3403	-3060	-3284	-2792	-1380	-2682	-2263	122
-	-149	-500	233	43	-381	399	106	-626	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											
119(R)	-1606	-2372	-2007	-936	-3104	-2201	-232	-2516	2216	-2240	-1550	-902	-2183	184	-1499	-1347	-2267	-2130	-1962	123
-	-149	-500	233	43	-381	399	106	-626	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											
120(C)	-1091	-3500	-3018	-1078	-3015	-3015	-2288	2503	-2770	-275	-25	-2674	-2983	-2523	-2739	-2210	-1074	2201	-2079	124
-	-149	-500	233	43	-381	399	106	-626	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											
121(Y)	-1005	-771	-3013	-2486	1856	-2498	-774	1186	-2131	1308	517	-1953	-2456	-1645	-1947	-1603	-935	12	-328	125
-	-149	-500	233	43	-381	399	106	-626	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

122(R)	-1618	-2368	-2015	-954	-3097	-2205	-244	-2514	1958	-2242	-1557	-915	-2192	170	3178	-1515	-1361	-2267	-2132	-1967	126
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

123(T)	-31	-658	-1594	-1483	-2301	-927	-1425	-1916	-1414	-2229	-1444	-1045	-1586	-1265	1349	3012	-1257	-2555	-2195	127	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

124(S)	-425	-1013	-1661	-1792	-2558	-1187	-1802	-2565	-1897	-2800	-2098	-1367	-1878	-1746	-1993	3270	-814	-1808	-2742	-2368	128
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

125(D)	-2059	-2867	-3860	-532	-3452	-1877	-1516	-3665	-1847	-3616	-3145	-882	-2414	-1318	-2469	-1872	-2198	-3236	-3167	-2847	129
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

126(V)	-1294	-1167	-3176	-3053	-1510	-2597	-2599	776	-2808	-665	-568	-2665	-2928	-2721	-2804	-2093	-1429	-3343	-2527	-2099	130
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

127(S)	1433	-625	-1729	-1673	-2531	-873	-1599	-2195	-1665	-2487	-1661	-1113	-1571	-1450	-1807	2786	-373	-1395	-2764	-2440	131
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

128(F)	-2605	-2164	-3410	-3518	-4186	-3034	-940	-1504	-3300	-1072	-1142	-2698	-3246	-2697	-2999	-2815	-2670	-1743	-274	806	132
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

129(M)	-778	2068	-3064	-2496	-565	-2504	-1497	1629	-2179	11	-2514	-2111	-2492	-1852	-2073	-1628	-733	2095	-1332	-989	133
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*
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130(A)	2576	-604	-1716	-1592	-2503	895	-1526	-2207	-1588	-2442	-1589	-1066	-1541	-1356	-1766	1089	-332	-1390	-2720	-2403	134
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

131(W)	1057	-423	-1396	-834	-306	-1603	1535	20	-598	-296	367	-808	-1690	-452	-791	-637	-225	1077	2854	-289	135
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

132(D)	-1299	-3082	2892	2152	-3290	-1299	-612	-3186	-788	-3089	-2397	95	-1806	-275	-1580	-987	-1355	-2689	-3263	-2321	136
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

133(A)	1980	-568	-1754	-1474	-1797	1737	-1271	-1167	-1349	-1645	-923	-1058	-1593	-1159	-1530	-274	-322	876	-2145	-1790	137
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

134(D)	1587	-2023	1916	376	-2494	-1219	-341	-2252	-201	-2265	-1443	1603	-1579	40	-787	-539	-712	-1798	-2489	-1753	138
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

135(H)	-380	-1637	-315	259	-1928	-1346	1589	-1609	1185	-1608	-749	1433	-1428	490	1250	-293	-304	142	-1806	-1220	139
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

136(L)	-2228	-1908	-3555	-3373	-604	-3255	-2515	-62	-2963	2976	358	-3207	-3275	-2644	-2802	-2988	-2232	-567	-1904	-1643	140
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

137(S)	-425	-1013	-1661	-1792	-2558	-1187	-1802	-2565	-1897	-2800	-2098	-1367	-1878	-1746	-1993	3270	-814	-1808	-2742	-2368	141
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-	-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

138(G)	-1814	-2025	-2478	-2748	-3577	-2700	-3847	-3052	-3864	-3354	-2492	-2699	-2874	-2984	-2040	-2187	-3148	-3081	-3439	142
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

139(S)	-425	-1013	-1661	-1792	-2558	-1187	-1802	-2565	-1997	-2800	-2098	-1367	-1878	-1746	-1993	-814	-1808	-2742	-2368	143
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

140(G)	-1814	-2025	-2478	-2748	-3577	-2700	-3847	-3052	-3864	-3354	-2492	-2699	-2874	-2984	-2040	-2187	-3148	-3081	-3439	144
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

141(I)	-1608	-1307	-3432	-3238	-1179	-3095	-2687	-2941	-191	-206	-2987	-3216	-2818	-2915	-2635	-1653	832	-2363	-1952	145
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

142(G)	1140	-633	-1604	-1487	-2567	-1485	-2286	-1518	-2503	-1638	-1021	-1538	-1291	-1723	1944	-340	-1439	-2760	-2429	146
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

143(I)	-1412	-1016	-3813	-3418	-1236	-3507	-3003	-3215	-130	-75	-3172	-3393	-3017	-3250	-2786	-1406	1884	-2614	-2221	147
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

144(G)	1810	-701	-1719	-1781	-2689	-1755	-2362	-1900	-2657	-1839	-1206	-1645	-1640	-2008	-299	-478	-1525	-2885	-2624	148
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

145(I)	-1412	-1016	-3813	-3418	-1236	-3507	-3003	-3215	-130	-75	-3172	-3393	-3017	-3250	-2786	-1406	1884	-2614	-2221	149
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

146(Q)	-1762	-2287	-1132	-1144	-2520	-2040	-1289	-2773	-740	-2625	-2183	-1302	-2433	4141	-881	-1765	-1840	-2519	-2457	-2076	150
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

147(S)	-425	-1013	-1661	-1792	-2558	-1187	-1802	-2565	-1897	-2800	-2098	-1367	-1878	-1746	-1993	3270	-814	-1808	-2742	-2368	151
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

148(K)	-1892	-2397	-1701	-1300	-3050	-2216	-948	-2829	3583	-2696	-2116	-1348	-2450	-619	78	-1865	-1815	-2562	-2460	-2321	152
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

149(G)	-1814	-2025	-2478	-2748	-3577	-3650	-2700	-3847	-3052	-3864	-3354	-2492	-2699	-2874	-2984	-2040	-2187	-3148	-3081	-3439	153
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

150(T)	-665	-1148	-2040	-2098	-2442	-1424	-1936	-1850	-1956	-2305	-1815	-1645	-2071	-1911	-2001	-909	3609	-1444	-2672	-2426	154
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

151(T)	-665	-1148	-2040	-2098	-2442	-1424	-1936	-1850	-1956	-2305	-1815	-1645	-2071	-1911	-2001	-909	3609	-1444	-2672	-2426	155
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

152(V)	-1396	-983	-3847	-3450	-1338	-3551	-3074	2356	-3271	-253	-163	-3204	-3424	-3095	-3321	-2828	-1387	-2847	-2706	-2287	156
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

153(I)	-1608	-1307	-3432	-3238	-1179	-3095	-2687	3374	-2941	-191	-206	-2987	-3216	-2818	-2915	-2635	-1653	832	-2363	-1952	157
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

154(H)	-2323	-2419	-1868	-1956	-1368	-2396	-5124	-3143	-1645	-2924	-2595	-1989	-2806	-1873	-1712	-2381	-2439	-2923	-1719	-935	158
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
155(Q)	-1762	-2287	-1132	-1144	-2520	-2040	-1289	-2773	-740	-2625	-2183	-1302	-2433	-4141	-881	-1765	-1840	-2519	-2457	-2076	159
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
156(K)	-1383	-2308	-1643	-684	-2977	-2064	-157	-2423	-2495	-2166	-1434	-724	-2050	1271	2417	-1265	-1155	-2139	-2093	-1856	160
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
157(D)	-1253	-2521	-3255	144	-3228	1167	-878	-3157	-1066	-3127	-2440	-203	-1918	-574	-1777	-1067	-1387	-2610	-3163	-2448	161
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
158(L)	-1096	-1358	-1544	-1152	-644	-2175	-905	-263	-552	-2080	225	-1234	-2259	2027	-699	-1367	-1047	-506	-1463	-1019	162
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
159(L)	519	-957	-510	761	-1084	-1377	-186	-643	18	-943	-162	-266	741	91	-375	-396	-256	-453	-1365	-873	163
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
160(P)	-2089	-2190	-2567	-2787	-3372	-2253	-2663	-3607	-2895	-3590	-3215	-2612	-24122	-2846	-2839	-2316	-2418	-3135	-2975	-3234	164
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
161(L)	-2228	-1908	-3555	-3373	-604	-3255	-2515	-62	-2963	-2976	358	-3207	-3275	-2644	-2802	-2988	-2232	-567	-1904	-1643	165
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*
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162(S)	-309	-1107	-487	-525	-2412	-1057	-961	-2282	-840	-2422	-1619	1332	-1628	-688	-1197	2732	-580	-1619	-2564	-2005	166
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

163(N)	-1468	-2069	-762	-997	-2634	-1768	-1516	-3059	-1508	-3127	-2580	4001	-2312	-1391	-1789	-1503	-1700	-2572	-2658	-2191	167
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

164(L)	-2228	-1908	-3555	-3373	-604	-3255	-2515	-62	-2963	-2976	358	-3207	-3275	-2644	-2802	-2988	-2232	-567	-1904	-1643	168
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

165(E)	-1877	-2708	-271	-3539	-3235	-1868	-1322	-3210	-1299	-3203	-2676	-849	-2342	-1076	-1736	-1723	-1961	-2857	-2976	-2640	169
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

166(L)	-2228	-1908	-3555	-3373	-604	-3255	-2515	-62	-2963	-2976	358	-3207	-3275	-2644	-2802	-2988	-2232	-567	-1904	-1643	170
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

167(F)	-2605	-2164	-3410	-3518	-4186	-3034	-940	-1504	-3300	-1072	-1142	-2698	-3246	-2697	-2999	-2815	-2670	-1743	-274	806	171
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

168(P)	-168	-833	-1242	-1249	-2494	-996	-1398	-2287	-1361	-2495	-1696	-974	-3089	-1209	-1577	1589	-518	-1540	-2679	-2284	172
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

169(Q)	-1762	-2287	-1132	-1144	-2520	-2040	-1289	-2773	-740	-2625	-2183	-1302	-2433	-1209	-881	-1765	-1840	-2519	-2457	-2076	173
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-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											
170(A)	3260	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											174
171(P)	-2089	-2190	-2557	-2787	-3372	-2253	-2663	-3607	-2895	-3590	-3215	-2612	4122	-2846	-2839	-2316	-2418	-3135	-2975	-3234
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											175
172(L)	-1534	-1220	-3652	-3169	-506	-3314	-2387	842	-2852	2266	595	-2942	-3144	-2433	-2752	-2537	-1497	1919	-1863	-1646
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											176
173(L)	-2228	-1908	-3555	-3373	-604	-3255	-2515	-62	-2963	2976	358	-3207	-3275	-2644	-2802	-2988	-2232	-567	-1904	-1643
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											177
174(T)	-687	-1734	1996	82	-2437	-1245	-620	-2077	-553	-2262	-1517	-192	-1688	-289	-1091	-639	2593	-1655	-2537	-1885
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											178
175(L)	-865	-1070	-1980	-1784	-992	639	-1400	-475	-1550	2482	-226	-1598	-2221	-1461	-1629	-1221	-995	-514	-1675	-1284
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											179
176(D)	-1277	-3051	2640	2426	-3262	-1297	-598	-3152	-756	-3057	-2356	97	-1797	-258	-1537	-970	-1330	-2657	-3231	-2297
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											180

177(T)	741	-572	-1785	-1337	-1017	-1495	-1012	1165	-1127	-673	-131	-1150	-1838	-980	-1309	-671	2444	264	-1540	-1155	181
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
178(Y)	-2952	-2203	-3627	-3744	2312	-3534	-52	-1936	-3354	-1436	-1429	-2308	-3446	-2368	-2918	-2769	-2850	-2068	674	4232	182
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
179(R)	-2176	-2415	-2504	-1923	-3054	-2403	-1269	-3053	-167	-2856	-2350	-1844	-2662	-997	3887	-2228	-2131	-2807	-2501	-2452	183
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
180(Q)	541	-1165	-629	-174	-1300	-1442	-271	-837	115	125	-362	-362	-1609	2716	-208	-502	-409	-670	-1574	-1069	184
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
181(I)	-1608	-1307	-3432	-3238	-1179	-3095	-2687	3474	-2941	-191	-206	-2987	-3216	-2818	-2915	-2635	-1653	832	-2363	-1952	185
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
182(G)	-1814	-2025	-2478	-2748	-3577	3650	-2700	-3847	-3052	-3864	-3354	-2492	-2699	-2874	-2984	-2040	-2187	-3148	-3081	-3439	186
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
183(K)	-1892	-2397	-1701	-1300	-3050	-2216	-948	-2829	3583	-2696	-2116	-1348	-2450	-619	78	-1865	-1815	-2562	-2460	-2321	187
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
184(N)	-1468	-2069	-762	-997	-2634	-1768	-1516	-3059	-1508	-3127	-2580	4001	-2312	-1391	-1789	-1503	-1700	-2572	-2658	-2191	188
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

185(A)	3260	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619	189
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

186(A)	3260	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619	190
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

187(R)	834	-2050	-746	884	-2527	-1656	-47	-2132	1493	-1981	-1160	-301	-1712	1458	2363	-712	-703	-1768	-2022	-1576	191
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

188(Y)	-2742	-2290	-3014	-3139	701	-3008	-615	-2258	-2836	-1879	-1835	-2399	-3220	-2421	-2640	-2733	-2781	-2314	18	4561	192
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

189(A)	3260	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619	193
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

190(K)	-1556	-2356	-1907	-881	-3072	-2167	-223	-2498	2928	-2228	-1530	-865	-2155	194	2195	-1447	-1308	-2240	-2125	-1942	194
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

191(K)	-871	-1938	-838	-231	-2459	1602	-133	-2052	1838	-1953	-1161	-399	-1768	286	1695	-786	-764	-1712	-2022	-1608	195
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

192(M)	-677	-1520	-268	2336	-1519	-1510	-393	-1012	-85	-1090	-2570	-321	-1724	-69	-467	-689	-653	-891	-1835	-1289	196
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

193(S)	-425	-1013	-1661	-1792	-2558	-1187	-1802	-2565	-1897	-2800	-2098	-1367	-1878	-1746	-1993	3270	-814	-1808	-2742	-2368	197
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
194(P)	-2089	-2190	-2567	-2787	-3372	-2253	-2663	-3607	-2895	-3590	-3215	-2612	4122	-2846	-2839	-2316	-2418	-3135	-2975	-3234	198
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
195(Q)	-305	-1517	-118	288	-1938	-1216	-55	-1644	319	-1680	-817	1055	-1400	1561	-150	1420	724	-1244	-1916	-1290	199
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
196(P)	-2089	-2190	-2567	-2787	-3372	-2253	-2663	-3607	-2895	-3590	-3215	-2612	4122	-2846	-2839	-2316	-2418	-3135	-2975	-3234	200
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
197(V)	-1294	-1167	-3176	-3053	-1510	-2597	-2599	776	-2808	-665	-568	-2665	-2928	-2721	-2804	-2093	-1429	3343	-2527	-2099	201
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
198(P)	-2089	-2190	-2567	-2787	-3372	-2253	-2663	-3607	-2895	-3590	-3215	-2612	4122	-2846	-2839	-2316	-2418	-3135	-2975	-3234	202
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
199(T)	-410	-679	-2187	-1873	-1334	-1555	-1509	252	-1620	-819	-414	-1498	-2017	-1493	-1731	-819	2683	1729	-1987	-1601	203
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
200(V)	-640	-504	-2456	-1869	-431	-2217	-1096	1416	-1525	927	467	-1651	-2226	-1310	527	-1301	-584	-2026	-1123	-778	204
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
201(N)	-1468	-2069	-762	-997	-2634	-1768	-1516	-3059	-1508	-3127	-2580	-4001	-2312	-1391	-1789	-1503	-1700	-2572	-2658	-2191	205
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
202(D)	-2059	-2867	-3860	-532	-3452	-1877	-1516	-3665	-1847	-3616	-3145	-882	-2414	-1318	-2469	-1872	-2198	-3236	-3167	-2847	206
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
203(Q)	-1762	-2287	-1132	-1144	-2520	-2040	-1289	-2773	-740	-2625	-2183	-1302	-2433	-1141	-881	-1765	-1840	-2519	-2457	-2076	207
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
204(M)	-1736	-1678	-2975	-2821	-903	-2619	-2137	-342	-2336	24	-2581	-2886	-2281	-2280	-2241	-1843	-654	-1928	-1574	-249	208
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
205(V)	2074	-651	-2390	-2110	-1415	-1553	-1686	337	-1896	-878	-474	-1620	-2049	-1709	-1970	-833	-621	-2210	-2098	-1727	209
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
206(R)	-2176	-2415	-2504	-1923	-3054	-2403	-1269	-3053	-167	-2856	-2350	-1844	-2662	-997	-3887	-2228	-2131	-2807	-2501	-2452	210
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
207(P)	-464	-829	-1870	-1666	-1484	-1471	-1433	-307	-1476	-1083	-664	-1375	-2672	-1386	-1610	-788	-689	1790	-2036	-1633	211
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
208(K)	-1185	-2213	-946	-394	-2739	-1847	-198	-2317	-2731	-2136	-1393	-533	-1934	2194	759	-1067	-1032	-2005	-2126	-1773	212

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*											

209(Y)	-2985	-2209	-3678	-3814	3177	-3561	-43	-1955	-3416	-1446	-1439	-2321	-3463	-2389	-2950	-2792	-2877	-2087	687	3771	213
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

210(Q)	-762	-1364	-955	-522	-1120	-1735	-477	-761	27	-599	2479	-672	-1881	-2972	-193	-868	-726	-767	-1578	-1080	214
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

211(A)	-3260	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619	215
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

212(K)	-1047	-1653	-1327	-700	-1605	-1930	-401	-1092	-2639	963	-645	-789	-2011	-70	384	-1110	-943	-1040	-1759	-1351	216
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

213(S)	2117	-614	-1759	-1672	-2492	-871	-1578	-2151	-1642	-2440	-1614	-1110	-1565	-1427	-1789	-2362	-359	-1366	-2728	-2406	217
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

214(A)	-3260	-995	-2154	-2261	-2604	-1267	-2060	-2035	-2233	-2502	-1934	-1631	-1961	-2058	-2240	-724	-871	-1499	-2817	-2619	218
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

215(L)	-1899	-1496	-4056	-3537	-330	-3780	-2667	1776	-3219	-2526	815	-3381	-3385	-2604	-3022	-3020	-1820	326	-1861	-1762	219
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

216(M)	-1774	-1423	-3895	-3310	2035	-3493	-2118	380	-3002	2012	3024	-3054	-3139	-2298	-2747	-2656	-1671	-186	-1400	-1154	220
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

217(H)	-2323	-2419	-1868	-1956	-1368	-2396	-5124	-3143	-1645	-2924	-2595	-1989	-2806	-1873	-1712	-2381	-2439	-2923	-1719	-935	221
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

218(I)	-1608	-1307	-3432	-3238	-1179	-3095	-2687	-3474	-2941	-191	-206	-2987	-3216	-2818	-2915	-2635	-1653	832	-2363	-1952	222
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

219(K)	-1892	-2397	-1701	-1300	-3050	-2216	-948	-2829	-3583	-2696	-2116	-1348	-2450	-619	78	-1865	-1815	-2562	-2460	-2321	223
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

220(E)	-1877	-2708	-271	-3539	-3235	-1868	-1322	-3210	-1299	-3203	-2676	-849	-2342	-1076	-1736	-1723	-1961	-2857	-2976	-2640	224
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

221(T)	-665	-1148	-2040	-2098	-2442	-1424	-1936	-1850	-1956	-2305	-1815	-1645	-2071	-1911	-2001	-909	3609	-1444	-2672	-2426	225
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

222(K)	-1892	-2397	-1701	-1300	-3050	-2216	-948	-2829	-3583	-2696	-2116	-1348	-2450	-619	78	-1865	-1815	-2562	-2460	-2321	226
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

223(H)	-815	-1622	-721	-285	-154	-1730	-3084	-1465	74	-1447	-801	-458	-1830	2002	-226	-776	-742	-1248	-656	1967	227
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

224(V)	-1294	-1167	-3176	-3053	-1510	-2597	-2599	776	-2808	-665	-568	-2665	-2928	-2721	-2804	-2093	-1429	3343	-2527	-2099	228
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
225(V)	-1294	-1167	-3176	-3053	-1510	-2597	-2599	776	-2808	-665	-568	-2665	-2928	-2721	-2804	-2093	-1429	3343	-2527	-2099	229
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
226(Q)	256	-702	-834	-278	-767	-1453	-225	-282	-122	-586	1554	-425	-1546	1737	-468	-439	956	664	-1117	-668	230
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
227(G)	-983	-2484	2007	425	-2938	2044	-519	-2776	-557	-2732	-1965	1439	-1705	-165	-1244	-772	-1048	-2274	-2930	-2091	231
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
228(K)	1193	-1766	-773	-153	-2230	-1586	-104	-1829	2318	-1796	-998	-334	-1685	310	1077	-652	-627	-1498	-1931	-1482	232
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
229(K)	167	-1748	4	798	-2054	-1243	41	-1782	1364	-1757	-869	1291	1129	480	-27	-235	-297	-1367	-1949	-1287	233
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
230(P)	1754	-768	-1572	-1540	-2436	-1010	-1540	-2025	-1554	-2338	-1594	-1128	3036	-1391	-1716	-364	-511	-1375	-2661	-2342	234
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												
231(Q)	-302	-1026	-515	1250	-1130	-1413	-130	540	130	-886	-174	-226	-1505	1404	-275	-384	-243	962	-1392	-883	235
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

232(E)	-520	-1863	141	2054	-2168	-1277	-101	-1881	266	-1884	-1038	55	-1487	1357	-204	-398	1225	-1490	-2100	-1438	236
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

233(L)	-1842	-1440	-4032	-3516	-373	-3743	-2662	2090	-3208	2373	771	-3347	-3371	-2616	-3022	-2980	-1767	435	-1886	-1773	237
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

234(H)	-414	-1810	-213	1423	-2160	-1330	1754	-1870	1213	-1798	-908	698	-1426	515	1275	-294	-339	-1451	-1937	-1321	238
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

235(V)	-1402	-985	-3864	-3467	-1340	-3570	-3094	2599	-3291	-250	-162	-3221	-3436	-3114	-3341	-2848	-1392	2675	-2716	-2300	239
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

236(N)	920	-1434	421	730	-1653	-1247	54	147	406	-1410	-563	950	-1354	465	-80	-191	655	-999	-1694	-1080	240
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6672	-7714	-894	-1115	-701	-1378	*	*												

237(F)	-544	-419	-2445	-1855	1863	-2055	-851	925	-1541	1449	674	-1547	-2075	-1212	-1484	287	-485	363	-744	-325	241
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-35	-5959	-7001	-894	-1115	-701	-1378	*	*												

238(N)	40	-510	-553	-161	-916	-1020	-206	-174	-45	-698	-25	304	-1303	21	-374	-144	811	1214	-1256	-787	242
-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
-	*	*	*	*	*	*	*	*	0												

TABLE 14

HMMER2.0 [2.3.2]
NAME gamma_exp_seqs
LENG 175
ALPH Amino
RF no
CS no
MAP yes
COM hmmbuild gamma_hmm3 gamma_exp_1
COM hmmscalibrate gamma_hmm3
NSEQ 8

DATE Fri Mar 30 18:50:16 2007
CKSUM 2849

XT -8455 -4 -1000 -1000 -8455 -4 -8455 -4
NULT -4 -8455
NULE 595 -1558 85 338 -294 453 -1158 197 249 902 -1085 -142 -21 -313 45 531 201 384 -1998 -644
EVD -145.815567 0.162883

HMM	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
	m->m	m->i	m->d	i->m	i->i	d->m	d->d	b->m	m->e											
	-263	*	-2585																	
1(M)	-2974	-2816	-4159	-4200	-2091	-3504	-3380	-1690	-3763	-1179	5175	-3864	-3864	-3670	-3585	-3468	-3151	-2065	-3012	-2758
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	-263	*											
2(S)	-714	-1508	-1330	-1230	-3093	-1545	-1557	-2841	-1413	-2974	-2115	2328	-2119	-1253	-1778	2548	824	-2105	-3194	-2709
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											
3(E)	-1483	-3123	1915	2354	-3400	-1775	-923	-3218	-893	-3153	-2360	-393	-2183	-548	-1565	-1244	1945	-2744	-3349	-2503
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-1162	-7442	-869	-894	-1115	-701	-1378	*	*											
4(I)	-1121	-753	-3426	-2999	-988	-3155	-2539	2556	-2786	50	147	-2768	-3061	-2590	-2831	-2397	-1109	2478	-2257	-1863
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-27	-6308	-7350	-894	-1115	-213	-2863	*	*											

5(D)	-683	-2011	~~2419~~ 66	-2272	-1583	-322	411	745	-2004	-1135	766	-1725	104	-470	-581	551	-1598	-2239	-1583	5
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											
6(D)	887	-1768	~~1716~~ 30	-2010	-1563	-265	-85	855	-1760	-908	-259	-1671	151	-387	-505	1220	-1340	-2037	-1419	6
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											
7(M)	-1176	-998	-3214	-2614	-619	-2754	-1611	1640	-2276	1581	~~2955~~ 913	-2724	-1911	-2174	-1847	-1112	-23	-1437	-1150	7
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											
8(E)	-548	-1816	-459	~~1276~~ -2069	-1544	-222	-1765	164	-1806	-936	1067	-1642	207	567	394	406	1270	-2054	-1423	8
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											
9(A)	~~2188~~ -871	-2463	-1968	-1209	-1970	-1409	1148	-1738	-981	-376	-1683	-2275	-1518	-1838	1104	-795	745	-1713	-1346	9
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-414	-7442	-2036	-894	-1115	-701	-1378	*	*											
10(M)	-1361	-2209	-1685	-799	-2653	-2166	-374	-2159	2416	-2060	~~2788~~ -871	-2171	32	1952	-1303	-1181	-1918	-2154	-1850	10
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-16	-7045	-8087	-894	-1115	-1546	-605	*	*											
11(U)	-1704	-1251	-4267	-3901	-1705	-3988	-3661	~~2754~~ -3767	-562	-486	-3661	-3816	-3619	-3839	-3300	-1696	2713	-3195	-2754	11
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-266	-7045	-2636	-894	-1115	-1546	-605	*	*											
12(R)	1194	-878	-1215	-668	-1215	-1507	-535	-506	-211	-969	-305	-716	-1730	-314	~~1868~~ -601	-426	1358	-1529	-1103	12
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*											
13(D)	1131	-2081	~~2040~~ 353	-2427	-1304	-271	-2177	-37	-2169	-1326	21	-1589	2011	-588	-528	-665	-1749	-2383	-1663	13
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*											
14(Q)	-741	-1209	-1130	-788	-1315	-1753	-757	-405	-373	-922	-481	-883	-1990	~~2875~~ -594	-942	-769	1796	-1762	-1264	14

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*											
15(L)	-1993	-1572	-4217	-3701	-440	-3917	-2829	2053	-3397	~~2455~~	714	-3538	-3506	-2756	-3185	-3166	-1913	308	-1987	-1900
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*											
16(Q)	-399	-1413	-310	-85	-2275	1203	-472	-2002	-154	-2063	-1220	-284	-1579	~~2043~~	-581	1618	-505	-1503	-2289	-1701
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-385	-6798	-2150	-894	-1115	-1832	-476	*	*											
17(R)	-710	-1458	-960	-581	-2313	1738	-519	-2033	255	-2052	-1310	-650	-1763	-179	~~2634~~	-756	-786	-1624	-2089	-1747
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-25	-6438	-7480	-894	-1115	-2111	-380	*	*											
18(M)	-369	-603	-1693	-1260	-653	-1556	-893	145	-936	-76	~~3276~~	-1117	-1837	-860	-1082	-738	1769	157	-1321	-946
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-25	-6438	-7480	-894	-1115	-1296	-754	*	*											
19(N)	-811	-2335	341	1694	-2622	-1349	-285	-2396	-5	-2331	-1491	~~2186~~	-1641	2048	-522	-624	-785	-1955	-2502	-1765
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*											
20(S)	1743	-708	-1922	-1872	-2675	-960	-1760	-2350	-1853	-2638	-1802	-1250	-1669	-1622	-1975	~~2720~~	-467	-1516	-2907	-2602
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*											
21(A)	~~1407~~	-647	-1573	-1206	-1183	1260	-962	-633	-1050	1273	-348	-1021	-1738	-901	-1248	-528	-406	-420	-1612	-1224
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*											
22(Q)	-943	-2552	1971	414	-2811	-1334	-397	-2628	-273	-2557	-1739	1988	-1696	~~2404~~	-888	-723	-941	-2164	-2737	-1929
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-247	-6798	-2753	-894	-1115	-1832	-476	*	*											
23(Q)	1213	-1833	1439	441	-2175	-1191	-112	-1906	160	-1918	-1072	120	-1444	~~2082~~	-368	-358	-468	-1496	-2144	-1452
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249

-	-23	-6573	-7616	-894	-1115	-1440	-663	*	*												
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24(G)	-78	-753	-1596	-1414	-2534	1905	-1434	-2239	-1404	-2450	-1598	-1042	-1626	-1216	-1645	1669	1888	-1468	-2725	-2364	24
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

25(T)	-290	-1158	-455	992	-1440	-1324	-182	-1023	92	-1236	-454	-204	-1490	162	-346	1073	1304	618	-1621	-1082	25
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

26(A)	-2848	-686	-2099	-2005	-2426	-1015	-1766	-1806	-1874	-2283	-1547	-1327	-1708	-1670	-1958	-344	1455	-1205	-2739	-2445	26
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

27(P)	-117	-814	-1355	-1082	-2287	-1034	-1151	-1960	-1028	-2169	-1343	-882	2175	-896	-1323	1590	1811	-1330	-2476	-2063	27
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

28(A)	-1889	-735	-1594	-1300	-2159	-1036	-1254	-1753	-1199	-2034	-1241	-997	1840	-1048	-1449	-299	1828	-1186	-2406	-2028	28
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

29(T)	1146	-812	-1308	-911	-2113	-1049	-968	-1778	-813	-1976	-1149	-782	1200	-689	-1149	1530	1750	-1213	-2300	-1865	29
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

30(A)	-2697	-693	-1984	-1936	-2645	-959	-1785	-2307	-1901	-2607	-1776	-1275	-1671	-1663	-2006	1734	-463	-1490	-2890	-2593	30
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

31(T)	1184	-788	-1393	-1051	-2205	1459	-1096	-1875	-979	-2081	-1251	-859	1250	-835	-1291	-265	1793	-1268	-2400	-1985	31
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

32(A)	2154	-729	-1615	-1319	-2152	-1035	-1263	-1746	-1215	-2029	-1236	-1006	1292	-1061	-1461	-298	1830	-1180	-2403	-2027	32
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												

33(T)	1265	-698	-1787	-1520	-2328	-990	-1421	-1963	-1411	-2231	-1411	-1092	-1625	-1232	-1627	1653	2477	-1298	-2566	-2217	33
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												
34(Q)	1091	-1611	-110	225	-2092	488	-167	-1806	180	-1836	-972	1294	-1480	1910	-301	-326	-396	-1385	-2066	-1429	34
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												
35(G)	-578	-1644	1459	30	-2499	1928	-604	-2224	-483	-2306	-1490	-223	-1659	-249	-1016	-545	1609	-1718	-2557	-1909	35
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												
36(Q)	807	-1248	-477	-5	-1851	-1220	-253	-1525	83	-1631	-790	-211	-1467	1893	-361	1196	1007	-1126	-1919	-1350	36
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												
37(H)	-351	-1466	-201	1372	-1675	-1321	2299	-1349	291	-1450	-624	-37	-1449	341	-175	687	-300	458	-1746	-1145	37
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												
38(R)	1090	-1963	-1013	-316	-2459	-1757	-168	-2034	1908	-1949	-1158	-475	-1824	253	2199	-834	-795	-1708	-2033	-1626	38
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1832	-476	*	*												
39(Q)	992	-1745	-25	1456	-2070	-1277	-45	-1793	323	-1793	-913	46	-1436	1490	-175	727	-353	-1385	-2006	-1343	39
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6798	-7840	-894	-1115	-1118	-891	*	*												
40(L)	595	-1237	1114	-159	-1422	-1497	-383	-998	-194	1266	-487	-383	615	-84	-625	-554	-467	-781	-1681	-1163	40
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7045	-8087	-894	-1115	-1546	-605	*	*												
41(Y)	-453	-1676	-399	153	-1937	616	-86	-1624	1122	-1661	-804	-125	-1534	338	985	905	-388	-1274	-1895	1329	41
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7045	-8087	-894	-1115	-1546	-605	*	*												

42(K)	858	-1517	-407	1108	-1738	-1452	-155	-1332	1346	-1489	-675	-178	-1563	238	-212	-417	-393	1080	-1811	-1232	42
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7045	-8087	-894	-1115	-348	-2223	*	*												
43(Q)	1257	-1903	-508	-123	-2463	-1568	-490	-2189	-125	-2201	-1319	1184	-1788	2352	-606	1081	-688	-1736	-2412	-1772	43
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
44(D)	-3359	-3855	4091	-1760	-4660	-2944	-2744	-5137	-3226	-4926	-4562	-2110	-3493	-2619	-3804	-3177	-3522	-4662	-4114	-4101	44
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
45(Y)	-4022	-3300	-4202	-4471	-162	-3952	-1522	-3554	-4217	-3007	-3045	-3501	-4179	-3602	-3853	-3928	-4069	-3631	-835	4786	45
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
46(P)	-3569	-3390	-4042	-4362	-4763	-3426	-4034	-5349	-4542	-5126	-4838	-4133	4267	-4442	-4297	-3840	-3922	-4781	-4041	-4662	46
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
47(L)	-3371	-2888	-4716	-4628	-1407	-4203	-3653	-947	-4306	3212	-381	-4472	-4191	-3767	-3993	-4264	-3364	-1600	-2791	-2682	47
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
48(A)	2566	-1673	-3241	-2895	1995	-2977	-836	-1285	-2608	-1350	-940	-2278	-3082	-2148	-2520	-2124	-1792	-1243	-329	2352	48
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
49(T)	556	-1927	-417	1122	-2324	-1539	-326	-2051	46	-2052	-1161	914	-1696	109	-456	1237	1507	-1625	-2264	-1607	49
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
50(K)	-1937	2062	-2451	-1377	-3415	-2629	-734	-2850	2929	-2646	-1935	-1362	-2625	-319	2284	-1860	-1722	-2588	-2595	-2389	50
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
51(H)	-1373	3432	-2086	-1398	-1984	-2275	3997	-1962	-97	-2063	-1380	-1376	-2458	-680	1376	-1482	-1351	-1730	-2090	-1576	51

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
52(P)	-877	-1501	-2346	-2559	-3585	-1679	-2607	-3503	-2784	-3706	-2880	-1979	3765	-2531	-2839	1007	-1303	-2500	-3737	-3472	52
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
53(E)	-2083	-3956	1757	3314	-4156	-1930	-1331	-4122	-1636	-3984	-3363	-536	-2485	-1014	-2522	-1729	-2166	-3591	-4126	-3126	53
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
54(W)	-918	-1893	-1053	-410	-2116	-1904	1403	-1750	939	191	-1025	-621	-1963	1572	1006	-890	-823	-1479	3807	-1561	54
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
55(I)	-2035	-1558	-4660	-4320	-2086	-4410	-4230	3075	-4225	-895	-832	-4094	-4207	-4109	-4326	-3753	-2029	2515	-3685	-3220	55
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
56(K)	-1221	-2380	-1220	-545	-2771	-2080	2222	-2412	2591	-506	-1493	-740	-2129	1828	236	-1118	-1094	-2072	-2372	-1913	56
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
57(T)	-638	-1254	-2731	-2815	-3380	-1515	-2573	-3085	-2761	-3390	-2550	-1961	-2264	-2507	-2764	1207	3489	-2165	-3623	-3371	57
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
58(P)	1508	-1457	-2747	-2921	-3587	-1692	-2758	-3293	-2991	-3584	-2799	-2133	3639	-2719	-2975	-1098	-1293	-2385	-3752	-3588	58
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
59(T)	-626	-1248	-2712	-2759	-3374	-1507	-2533	-3087	-2703	-3377	-2628	-1935	-2251	-2449	-2729	2022	3167	-2161	-3609	-3353	59
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
60(G)	-1416	-2145	-1208	-1507	-3831	3184	-2206	-3881	-2367	-3956	-3204	2058	-2622	-2014	-2725	-1515	-1765	-2999	-3822	-3422	60
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
61(K)	-3217	-3413	-3082	-2664	-4291	-3257	-2104	-4231	3883	-3955	-3433	-2635	-3520	-1813	-964	-3215	-3130	-3953	-3447	-3538	61
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
62(N)	997	-1468	-1225	-894	-2636	-1532	-1137	-2328	-901	-2462	-1613	2219	1916	-793	-1309	-715	1524	-1770	-2737	-2223	62
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
63(L)	-2522	-2108	-4820	-4349	-929	-4400	-3462	148	-4047	2807	234	-4148	-4027	-3348	-3792	-3703	-2466	1342	-2528	-2466	63
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
64(T)	-788	-2299	1480	1207	-2603	-1587	-371	-2366	1056	-2307	-1406	1363	-1766	69	-565	-641	1533	-1914	-2480	-1766	64
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
65(D)	-2128	-4054	3436	1917	-4232	-1928	-1353	-4214	-1706	-4066	-3470	-529	-2498	-1042	-2637	-1757	-2219	-3673	-4218	-3179	65
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
66(I)	-2331	-1899	-4695	-4181	1172	-4286	-3041	3269	-3918	981	175	-3898	-3911	-3237	-3671	-3510	-2255	6	-2249	-1928	66
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
67(T)	-1680	-2119	-3327	-3541	-3740	-2348	-3223	-3396	-3447	-3750	-3249	-2849	-3039	-3332	-3347	-1934	3920	-2813	-3757	-3736	67
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
68(L)	-3371	-2888	-4716	-4628	-1407	-4203	-3653	-947	-4306	3212	-381	-4472	-4191	-3767	-3993	-4264	-3364	-1600	-2791	-2682	68
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
69(E)	-2083	-3956	1757	3314	-4156	-1930	-1331	-4122	-1636	-3984	-3363	-536	-2485	-1014	-2522	-1729	-2166	-3591	-4126	-3126	69
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

70(N)	667	-2223	-552	-466	-2986	-1791	-914	-2689	705	-2706	-1900	3410	-2148	-538	-805	-1078	-1201	-2222	-2879	-2293	70
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
71(N)	-2174	-1982	-4186	-4170	-2484	-3406	-3713	42	-3993	-1584	-1517	-3697	-3771	-3891	-3924	-3057	-2329	3646	-3507	-3148	71
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
72(L)	-2729	-2229	-5089	-4576	-885	-4785	-3686	2209	-4314	2611	306	-4471	-4194	-3468	-3998	-4097	-2634	-271	-2572	-2589	72
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
73(N)	956	-2274	1213	-94	-2844	-1631	-685	-2609	-449	-2590	-1722	2259	-1939	-278	-1001	1526	-973	-2118	-2789	-2080	73
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
74(G)	-1785	-3613	2207	1292	-3820	2213	-1112	-3710	-1261	-3595	-2872	1221	-2322	-764	-2053	-1476	-1833	-3198	-3789	-2827	74
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
75(K)	-708	-2181	1118	746	-2508	-1590	-284	-2254	1976	-2187	-1279	1149	-1720	166	1307	-575	-648	-1808	-2344	-1667	75
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
76(N)	-2021	-1555	-4630	-4292	-2072	-4355	-4168	1922	-4183	-894	-829	-4054	-4177	-4066	-4278	-3696	-2019	3287	-3647	-3180	76
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
77(K)	-812	-2228	1238	34	-2594	1219	-435	-2340	1781	-2306	-1421	-281	-1809	-4	-596	-685	1266	-1898	-2493	-1804	77
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
78(A)	2961	-1382	-2807	-2930	-3515	-1634	-2710	-3207	-2947	-3502	-2695	-2093	2243	-2664	-2935	-1021	-1211	-2301	-3704	-3525	78
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

79(Q)	-967	-2422	-345	1056	-2767	-1728	-447	-2497	1288	-2409	-1534	1390	-1900	2852	-350	-818	-905	-2063	-2544	-1892	79
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
80(D)	-3359	-3855	4091	-1760	-4660	-2944	-2744	-5137	-3226	-4926	-4562	-2110	-3493	-2619	-3804	-3177	-3522	-4662	-4114	-4101	80
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
81(M)	-2091	-1713	-4451	-3905	-954	-3989	-2968	336	-3615	1206	3494	-3630	-3713	-3048	-3417	-3183	-2024	2361	-2329	-2191	81
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
82(R)	-3572	-3491	-3930	-3374	-4367	-3463	-2544	-4583	-1435	-4243	-3795	-3226	-3764	-2331	4134	-3660	-3534	-4308	-3567	-3769	82
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
83(I)	-2506	-2132	-4383	-4301	-2107	-3870	-3753	3806	-4080	-1081	-1125	-4012	-4038	-3936	-3993	-3643	-2570	78	-3292	-2940	83
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
84(T)	-624	-1248	-2705	-2744	-3377	-1505	-2523	-3093	-2688	-3379	-2528	-1928	-2247	-2435	-2721	2328	2957	-2163	-3609	-3352	84
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
85(P)	-1686	-2611	-1672	-1050	-3249	-2373	-759	-2812	341	-2647	-1909	-1144	2832	1103	2390	-1606	-1559	-2494	-2639	-2322	85
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
86(D)	737	-2727	2071	1949	-3005	-1688	-649	-2797	-446	-2728	-1869	-302	-1983	1707	-1039	-948	-1120	-2333	-2908	-2133	86
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
87(T)	-1680	-2119	-3327	-3541	-3740	-2348	-3223	-3396	-3447	-3750	-3249	-2849	-3039	-3332	-3347	-1934	3920	-2813	-3757	-3736	87
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
88(L)	-3371	-2888	-4716	-4628	-1407	-4203	-3653	-947	-4306	3212	-381	-4472	-4191	-3767	-3993	-4264	-3364	-1600	-2791	-2682	88

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
89(R)	-1604	-2727	-1441	2175	-3300	-2312	-612	-2841	1592	-2626	-1849	-957	-2366	-182	2492	-1470	-1439	-2503	-2603	-2246	89
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
90(M)	-2061	-1752	-4123	-3574	-361	-3659	-2041	-262	-3197	2031	3098	-3161	-3441	-2603	-2964	-2802	-1970	-720	-1446	2614	90
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
91(Q)	-3221	-3397	-2531	-2668	-3852	-3172	-2736	-4443	-2278	-4160	-3817	-2785	-3623	4473	-2343	-3268	-3360	-4139	-3574	-3442	91
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
92(A)	2875	-1335	-2928	-3126	-3684	2067	-2851	-3441	-3216	-3720	-2847	-2129	-2353	-2834	-3138	-970	-1176	-2380	-3856	-3729	92
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
93(Q)	333	-2205	-255	1818	-2553	-1603	-404	-2298	-46	-2265	-1377	-266	-1788	2587	-554	426	-748	-1861	-2452	-1766	93
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
94(I)	-2036	-1562	-4657	-4316	-2070	-4401	-4214	3210	-4217	-878	-820	-4089	-4201	-4095	-4314	-3744	-2031	2315	-3666	-3206	94
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
95(A)	3527	-1872	-3386	-3637	-3817	-2114	-3249	-3478	-3634	-3846	-3228	-2714	-2847	-3362	-3481	-1630	-1813	-2725	-3849	-3859	95
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
96(E)	657	-2144	-303	1992	-2460	-1564	-271	-2208	916	-2153	-1243	1296	-1696	1197	-360	-543	-616	-1765	-2321	-1636	96
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
97(D)	-1428	-3090	2456	0	-3358	626	-868	-3185	-800	-3101	-2290	1589	-2147	2323	-1457	-1192	-1436	-2703	-3285	-2444	97
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*
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98(M)	2458	-1063	-2439	-2109	-2009	-1619	-1748	-1532	-1906	-1860	-1662	-2171	-1718	-2036	877	-861	-1187	-2405	-2053	98
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											

99(G)	-1362	-2477	-504	-475	-2812	-2719	1818	-2783	-476	-2759	-1994	-738	-2262	1773	-798	-1267	-1389	-2373	-2786	-2136	99
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

100(R)	-3572	-3491	-3930	-3374	-4367	-3463	-2544	-4583	-1435	-4243	-3795	-3226	-3764	-2331	-134	-3660	-3534	-4308	-3567	-3769	100
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

101(H)	-810	2146	2140	-126	-2275	-1646	-2820	-2018	-274	-2093	-1264	-413	982	-165	-770	-747	-802	-1656	-2340	-1698	101
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

102(A)	-1710	-2315	-2315	-1954	-3110	-1911	-1672	-2739	-942	-2886	-2159	-1698	-2440	-1397	1826	-1250	-1338	-2189	-3054	-2761	102
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

103(I)	-2053	-1588	-4631	-4216	-1683	-4330	-3782	-2657	-4066	1111	-476	-3979	-4072	-3758	-4047	-3615	-2023	-2344	-3177	-2869	103
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

104(A)	-1741	-1377	-1306	-2967	-1773	-1543	-2675	-1182	-2815	-2087	-1302	-2305	2631	-1425	-1113	-1241	-2132	-3062	-2602	-249	104
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

105(R)	-2019	-2443	-2872	-1909	-2475	-2767	-1140	-2083	30	-1845	2733	-1796	-2859	-814	-2113	-1903	-2079	-2488	-2193	-249	105
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

106(N)	-2749	-3127	-2054	-2376	-3897	-2856	-2871	-4637	-3059	-4563	-4104	4298	-3433	-2867	-3263	-2819	-3038	-4043	-3716	-3493	106
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
107(F)	-3172	-2615	-4595	-4495	4044	-4307	-1507	-1008	-4122	717	-475	-3498	-4094	-3206	-3712	-3641	-3094	-1537	-738	314	107
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
108(R)	-1406	-2631	-1037	1390	-3118	-2127	-576	-2728	338	-2561	-1757	-770	-2229	2482	2529	-1268	-1281	-2363	-2589	-2141	108
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
109(R)	-3572	-3491	-3930	-3374	-4367	-3463	-2544	-4583	-1435	-4243	-3795	-3226	-3764	-2331	4134	-3660	-3534	-4308	-3567	-3769	109
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
110(A)	3527	-1872	-3386	-3637	-3817	-2114	-3249	-3478	-3634	-3846	-3228	-2714	-2847	-3362	-3481	-1630	-1813	-2725	-3849	-3859	110
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
111(A)	2600	-1202	-2867	-2874	-3511	640	-2600	-3272	-2839	-3509	-2602	-1948	-2213	-2506	-2861	2031	-1000	-2218	-3720	-3512	111
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
112(E)	-3263	-3764	-1446	3823	-4534	-2947	-2635	-4813	-2809	-4652	-4247	-2114	-3462	-2477	-3224	-3106	-3391	-4409	-4008	-3980	112
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
113(L)	-3371	-2888	-4716	-4628	-1407	-4203	-3653	-947	-4306	3212	-381	-4472	-4191	-3767	-3993	-4264	-3364	-1600	-2791	-2682	113
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

114(T)	-1377	-1356	-3473	-3179	-1732	-2771	-2620	2590	-2866	-935	-728	-2732	-3097	-2717	-2873	-2060	2702	379	-2680	-2298	114
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
115(A)	2529	-1216	-2947	-3039	-3523	-1479	-2704	-3262	-2994	-3544	-2659	-2017	-2245	-2654	-2953	2488	-1036	-2225	-3753	-3552	115
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
116(I)	-2035	-1558	-4660	-4320	-2086	-4410	-4230	3075	-4225	-895	-832	-4094	-4207	-4109	-4326	-3753	-2029	2515	-3685	-3220	116
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
117(P)	-877	-1501	-2346	-2559	-3585	-1679	-2607	-3503	-2784	-3706	-2880	-1979	3765	-2531	-2839	1007	-1303	-2500	-3737	-3472	117
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
118(D)	-3359	-3855	4091	-1760	-4660	-2944	-2744	-5137	-3226	-4926	-4562	-2110	-3493	-2619	-3804	-3177	-3522	4662	-4114	-4101	118
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
119(D)	1000	-3403	2884	1734	-3674	-1814	-1070	-3527	-1163	-3445	-2700	-428	-2286	-716	-1910	-1407	-1728	-3031	-3645	-2731	119
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
120(R)	-1763	-2178	-2815	-2009	-2626	-2577	-1312	-1725	-215	-2141	-1691	-1848	-2804	-1008	3553	-1932	-1770	1073	-2627	-2315	120
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
121(I)	-2084	-1616	-4657	-4243	-1653	-4365	-3815	3132	-4093	890	-443	-4016	-4093	-3767	-4069	-3656	-2054	1752	-3174	-2884	121
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

122(L)	-3371	-2888	-4716	-4628	-1407	-4203	-3653	-947	-4306	-381	-4472	-4191	-3767	-3993	-4264	-3364	-1600	-2791	-2682	122
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											

123(E)	100	-3437	1680	-3073	-3701	-1819	-1079	-3559	-1178	-3473	-2732	-430	-2294	-725	-1928	-1421	-1748	-3060	-3669	-2750	123
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

124(I)	-2462	-2009	-4861	-4316	-906	-4477	-3404	-3063	-4059	1320	2522	-4133	-4007	-3310	-3785	-2374	-100	-2484	-2465	124	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

125(Y)	-4022	-3300	-4202	-4471	-162	-3952	-1522	-3554	-4217	-3007	-3045	-3501	-4179	-3602	-3853	-3928	-4069	-3631	-835	-4786	125
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

126(N)	-2749	-3127	-2054	-2376	-3897	-2856	-2871	-4637	-3059	-4563	-4104	-4298	-3433	-2867	-3263	-2819	-3038	-4043	-3716	-3493	126
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

127(A)	-1216	-2991	-3125	-3530	-1484	-2756	-3260	-3083	-3557	-2679	-2048	-2256	-2728	-3008	1226	-1046	-2226	-3770	-3578	127	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

128(L)	-3371	-2888	-4716	-4628	-1407	-4203	-3653	-947	-4306	-381	-4472	-4191	-3767	-3993	-4264	-3364	-1600	-2791	-2682	128	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

129(R)	-3572	-3491	-3930	-3374	-4367	-3463	-2544	-4583	-1435	-4243	-3795	-3226	-3764	-2331	-3660	-3534	-4308	-3567	-3769	129	
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

130(P)	-3569	-3390	-4042	-4362	-4763	-3426	-4034	-5349	-4542	-5126	-4838	-4133	4267	-4442	-4297	-3840	-3922	-4781	-4041	-4662	130
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
131(N)	-4048	-3064	-4528	-4828	3028	-4400	-683	-2944	-4397	-2302	-2372	-3069	-4275	-3191	-3805	-3657	-3917	-3103	65	4127	131
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
132(R)	-3572	-3491	-3930	-3374	-4367	-3463	-2544	-4583	-1435	-4243	-3795	-3226	-3764	-2331	4134	-3660	-3534	-4308	-3567	-3769	132
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
133(S)	-1298	-1844	-2875	-3136	-3678	-2026	-2995	-3857	-3302	-4033	-3306	-2458	-2758	-3055	-3253	3563	-1733	-2892	-3744	-3541	133
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
134(T)	-628	-1249	-2717	-2772	-3374	-1509	-2541	-3085	-2715	-3378	-2532	-1940	-2253	-2461	-2737	1826	3269	-2161	-3611	-3356	134
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
135(K)	-781	-1702	-906	-326	356	-1807	-409	-1467	2476	-1586	-831	-553	-1886	1683	-224	-780	-706	240	-1907	-1366	135
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
136(A)	1793	-2317	-182	1436	-2630	-1625	-446	-2381	-113	-2343	-1462	1229	-1828	1588	-633	-720	-823	-1946	-2530	-1828	136
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
137(E)	-3263	-3764	-1446	3823	-4534	-2947	-2635	-4813	-2809	-4652	-4247	-2114	-3462	-2477	-3224	-3106	-3391	-4409	-4008	-3980	137
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

138(L)	-3371	-2888	-4716	-4628	-1407	-4203	-3653	-947	-4306	3212	-381	-4472	-4191	-3767	-3993	-4264	-3364	-1600	-2791	-2682	138
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

139(L)	-2369	-2273	-3168	-2905	-984	-3520	-2341	-606	-2150	2925	-5	-2869	-3499	773	-2144	-2905	-2325	-1156	-2222	-1911	139
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

140(A)	-2169	1557	-918	-3547	-1815	-1738	-3268	-1907	-3465	-2733	-1101	-2422	-1474	-2446	-1331	-1572	-2621	-3648	-3069	-249	140
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

141(I)	-1583	-1435	-3802	-3522	-1810	-3145	-2999	-3393	-3226	-885	-741	-3089	-3390	-3083	-3224	-2453	1116	598	-2920	-2528	141
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

142(A)	-1216	-2991	-3125	-3174	-3530	-1484	-2756	-3260	-3083	-3557	-2679	-2048	-2256	-2728	-3008	1226	-1046	-2226	-3770	-3578	142
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

143(D)	785	-2776	-3038	-83	-3174	-1756	-838	-2947	1206	-2909	-2094	-427	-2118	-452	-1226	-1122	-1320	-2485	-3100	-2339	143
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

144(E)	-2080	-3966	2020	-3228	-4159	-1924	-1325	-4125	-1633	-3985	-3364	-528	-2480	-1008	-2526	-1723	-2163	-3592	-4133	-3124	144
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

145(L)	-3371	-2888	-4716	-4628	-1407	-4203	-3653	-947	-4306	3212	-381	-4472	-4191	-3767	-3993	-4264	-3364	-1600	-2791	-2682	145
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

146(E)	-1474	-2672	-1152	2693	-3190	-2187	-590	-2777	1538	-2592	-1796	-829	-2275	-160	1592	-1335	-1336	-2418	-2601	-2183	146
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
147(H)	-854	-2355	1981	1100	-2650	-1609	-2785	-2413	-120	-2363	-1474	-237	-1812	2	-665	423	558	-1968	-2546	-1827	147
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
148(K)	-1846	-2756	-2163	-1201	-3441	-2548	-678	-2892	-2570	-2658	-1923	-1238	-2547	-254	2397	-1742	1144	-2602	-2599	-2357	148
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
149(Y)	-4032	-3074	-4494	-4762	743	-4354	-719	-3014	-4275	-2384	-2440	-3079	-4257	-3186	-3737	-3658	-3908	-3152	3572	4429	149
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
150(H)	-1438	-2996	1391	-75	-3124	-1810	-3844	-3065	-667	-2987	-2193	-429	-2172	2489	-1215	-1218	-1432	-2622	-3084	-2281	150
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
151(A)	-3527	-1872	-3386	-3637	-3817	-2114	-3249	-3478	-3634	-3846	-3228	-2714	-2847	-3362	-3481	-1630	-1813	-2725	-3849	-3859	151
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
152(P)	-1025	-2197	-1034	-410	-2631	-1925	-444	-2266	1763	-2207	-1375	-627	1861	-14	1096	-940	1601	-1905	-2333	-1837	152
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												
153(V)	-2029	-1557	-4631	-4241	-1861	-4362	-3936	2290	-4115	428	-638	-4011	-4123	-3892	-4149	-3664	-2008	-2918	-3374	-2999	153
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	

-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*
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154(C)	-1181	4220	-2589	-2685	-3120	-1960	-2546	-3057	-2657	-3393	-2695	3434	-2639	-2541	-2713	-1406	-1561	-2401	-3333	-2954	154
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

155(A)	3520	-1872	-3386	-3637	-3817	-2114	-3249	-3478	-3634	-3846	-3228	-2714	-2847	-3362	-3481	-1630	-1813	-2725	-3849	-3859	155
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

156(G)	2090	-1967	1316	-759	-3390	-2191	-1492	-3161	-1542	-3255	-2440	-931	-2229	-1185	-2076	-1075	-1294	-2455	-3474	-2861	156
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

157(W)	-3904	-3011	-4520	-4764	3780	-4316	-778	-2763	-4325	-2135	-2197	-3117	-4228	-3209	-3773	-3640	-3795	-2959	-4425	1145	157
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

158(F)	-1811	-1421	-4284	-3810	-2418	-3829	-2928	2106	-3571	-448	-351	-3460	-3684	-3221	-3480	-3037	-1775	2416	-2494	-2056	158
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

159(R)	735	-2333	-863	1128	-2835	-1951	-604	-2472	145	-2416	-1604	-687	-2116	-188	-2967	-1082	-1113	-2097	-2537	-2033	159
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

160(E)	-3263	-3764	-1446	-3823	-4534	-2947	-2635	-4813	-2809	-4652	-4247	-2114	-3462	-2477	-3224	-3106	-3391	-4409	-4008	-3980	160
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

161(A)	3142	-1216	-2990	-3120	-3529	-1483	-2753	-3260	-3078	-3556	-2677	-2046	-2255	-2723	-3005	1291	-1045	-2226	3768	-3576	161
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											

162(A)	-1872	-3386	-3637	-3817	-2114	-3249	-3478	-3634	-3846	-3228	-2714	-2847	-3362	-3481	-1630	-1813	-2725	-3849	-3859	162
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											

163(E)	-1353	-2969	1558	-3260	316	-827	-3072	-733	-3001	-2179	-361	-2110	-438	-1376	-1133	601	-2596	-3191	-2371	163
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											

164(V)	-770	-686	-2514	-1945	-447	-2242	-1009	-25	-1638	907	143	1079	-2296	-1373	-1314	-714	-2075	-994	1702	164
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											

165(Y)	-4022	-3300	-4202	-4471	-162	-3952	-1522	-3554	-4217	-3007	-3045	-3501	-4179	-3602	-3853	-3928	-4069	-3631	-835	165
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											

166(E)	-538	-1578	-594	-1749	-1591	-271	182	64	-466	-709	-313	-1679	970	667	560	477	169	-1859	-1283	166
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*											

167(Q)	-583	-1987	-408	1304	-2289	-1544	-207	-2017	1086	-1990	-1087	-190	-1644	-2012	-258	724	522	445	-2178	-1519	167
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

168(R)	-2066	-2822	-1934	-1456	-3549	-2571	-1068	-3277	108	-3049	-2371	1548	-2762	-689	-3583	-2003	-1971	-2938	-2872	-2605	168
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

169(K)	-963	-2461	926	23	-2770	-1679	1437	-2530	2489	-2458	-1580	1750	-1887	-51	-577	-804	-916	-2086	-2613	-1914	169
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

170(K)	-3217	-3413	-3082	-2664	-4291	-3257	-2104	-4231	3883	-3955	-3433	-2635	-3520	-1813	-964	-3215	-3130	-3953	-3447	-3538	170
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

171(L)	-2562	-2146	-4846	-4375	-908	-4427	-3477	110	-4068	2853	256	-4182	-4041	-3350	-3803	-3735	-2504	1163	-2519	-2467	171
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

172(K)	-2474	-3104	-3210	-1788	-4002	-2957	-830	-3301	2947	-2931	-2275	-1649	-2892	-412	2812	-2351	-2137	-3082	-2748	-2679	172
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

173(G)	-1037	-1749	-1285	-849	-2174	2283	-841	794	1846	-1893	-1188	-977	-2185	-518	-441	-1114	-1045	-1375	-2263	-1816	173
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

174(D)	-1939	-3820	3052	1863	-4026	1028	-1223	-3952	-1477	-3823	-3155	-460	-2399	-893	-2340	-1601	-2010	-3421	-4014	-3000	174
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-12	-7442	-8484	-894	-1115	-701	-1378	*	*												

175(N)	355	-2563	2286	-73	-3075	-1686	-794	-2864	-638	-2825	-1980	2330	-2039	-399	-1233	944	-1176	-2366	-3020	-2262	175
-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
-	*	*	*	*	*	*	*	*	0												

CLAIMS

What is claimed is:

1. A recombinant microbial host cell comprising at least one DNA molecule encoding a polypeptide that catalyzes a substrate to product conversion selected from the group consisting of:

- i) pyruvate to alpha-acetolactate;
- ii) alpha-acetolactate to acetoin;
- iii) acetoin to 2,3-butanediol;
- iv) 2,3-butanediol to 2-butanone; and
- v) 2-butanone to 2-butanol;

wherein the at least one DNA molecule is heterologous to said microbial host cell and wherein said microbial host cell produces 2-butanol.

2. A recombinant microbial host cell comprising at least one DNA molecule encoding a polypeptide that catalyzes a substrate to product conversion selected from the group consisting of:

- i) pyruvate to alpha-acetolactate;
- ii) alpha-acetolactate to acetoin;
- iii) acetoin to 2,3-butanediol; and
- iv) 2,3-butanediol to 2-butanone;

wherein the at least one DNA molecule is heterologous to said microbial host cell and wherein said microbial host cell produces 2-butanone.

3. A host cell according to claims 1 or 2 wherein the polypeptide that catalyzes a substrate to product conversion of pyruvate to alpha-acetolactate is acetolactate synthase.

4. A host cell according to claims 1 or 2 wherein the polypeptide that catalyzes a substrate to product conversion of alpha-acetolactate to acetoin is acetolactate decarboxylase.

5. A host cell according to claims 1 or 2 wherein the polypeptide that catalyzes a substrate to product conversion of acetoin to 2,3-butanediol is butanediol dehydrogenase.
6. A host cell according to claims 1 or 2 wherein the polypeptide that catalyzes a substrate to product conversion of 2,3-butanediol to 2-butanone is diol dehydratase or glycerol dehydratase.
7. A host cell according to claim 1 wherein the polypeptide that catalyzes a substrate to product conversion of 2-butanone to 2-butanol is butanol dehydrogenase.
8. A host cell according to claims 1 or 2 wherein the cell is selected from the group consisting of: a bacterium, a cyanobacterium, a filamentous fungus and a yeast.
9. A host cell according to claim 8 wherein the cell is a member of a genus selected from the group consisting of *Clostridium*, *Zymomonas*, *Escherichia*, *Salmonella*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Alcaligenes*, *Klebsiella*, *Paenibacillus*, *Arthrobacter*, *Corynebacterium*, *Brevibacterium*, *Pichia*, *Candida*, *Hansenula* and *Saccharomyces*.
10. A host cell according to claim 3 wherein the acetolactate synthase has an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:77, and SEQ ID NO:79 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.
11. A host cell according to claim 4 wherein the acetolactate decarboxylase has an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID

NO:2, SEQ ID NO: 81, and SEQ ID NO:83 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

12. A host cell according to claim 5 wherein the butanediol dehydrogenase has an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:85, SEQ ID NO:87, and SEQ ID NO:89 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

13. A host cell according to Claim 6 wherein the diol dehydratase or glycerol dehydratase comprise full length large, medium and small subunits that each give an E-value parameter of 0.01 or less when queried using a Profile Hidden Markov Model prepared using the large subunits of SEQ ID NOs: 8, 99, 105, 135, 138, 141, 146, and 164; the medium subunits of SEQ ID NOs: 10, 101, 107, 136, 139, 142, 148, and 165; and the small subunits of SEQ ID NOs: 12, 103, 109, 137, 140, 143, 150, and 166; each query being carried out using the hmmsearch algorithm wherein the Z parameter is set to 1 billion.

14. A host cell according to Claim 6 wherein the diol dehydratase or the glycerol dehydratase identified by a process comprising the steps of:

- a) generating a Profile Hidden Markov Model from the alignment of the amino acid sequences corresponding to the large, medium and small subunits of diol and glycerol dehydratase enzymes wherein;
- i) the large subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 99, 105, 135, 138, 141, 146, and 164;

- ii) the medium subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 101, 107, 136, 139, 142, 148, and 165; and
 - iii) the small subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 103, 109, 137, 140, 143, 150, and 166;
- b) querying at least one public database of protein sequences containing sequences of diol and glycerol dehydratases with the Profile Hidden Markov Model of (a) using the hmmsearch algorithm wherein the Z parameter is set to 1 billion and the E-value parameter is set to 0.01, to identify a first data set of diol and glycerol dehydratase amino acid sequences; and
 - c) removing any partial sequences from the first data set of (b) to generate a second data set of diol and glycerol dehydratase amino acid sequences, wherein diol dehydratase and the glycerol dehydratase enzymes are identified.

15. A host cell according to Claim 6 wherein the diol dehydratase or glycerol dehydratase comprises a large subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 93, 99, 105, 135, 138, 141, 146, 164, 167, 170, 173, 176, 179, 182, 185, 188, 191, 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 130, 243, 254, 255, 256, 257, 258 and 259, based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

16. A host cell according to Claim 6 wherein the diol dehydratase or glycerol dehydratase comprises a medium subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 95, 101, 107, 136, 139, 142, 148, 165, 168, 171, 174, 177, 180, 183, 186, 189, 192, 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 244, 250,

252, 260, 261, 262, 263, 364, 265, 266, and 167 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

17. A host cell according to Claim 6 wherein the diol dehydratase or glycerol dehydratase comprises a small subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 97, 103, 109, 137, 140, 143, 150, 166, 169, 172, 175, 178, 181, 184, 187, 190, 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 234, 236, 238, 240, 242, 245, 248, 249, 251, 253, 268, 270, 271, 272, 273, and 274, based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

18. A host cell according to Claim 6 wherein the diol dehydratase or glycerol dehydratase comprises fused large, medium and small subunits comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 233, 235, 237, 239, 241, 246, and 247, based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

19. A host cell according to Claim 6 wherein the diol dehydratase or glycerol dehydratase comprises fused large, medium and small subunits and has at least 95% identity to an amino acid sequence comprising all three of the amino acid sequences encoding large, medium and small subunits, selected from the group consisting of:

- a) SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12;
- b) SEQ ID NO:93, SEQ ID NO:95, and SEQ ID NO:97;
- c) SEQ ID NO:99, SEQ ID NO:101, and SEQ ID NO:103;
- d) SEQ ID NO:105, SEQ ID NO:107, and SEQ ID NO:109;

- e) SEQ ID NO:135, SEQ ID NO:136, and SEQ ID NO:137;
- f) SEQ ID NO:138, SEQ ID NO:139, and SEQ ID NO:140;
- g) SEQ ID NO:146, SEQ ID NO:148, and SEQ ID NO:150;
- h) SEQ ID NO:141, SEQ ID NO:142, and SEQ ID NO:143; and
- i) SEQ ID NO:164, SEQ ID NO:165, and SEQ ID NO:166;

based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

20. A host cell according to claim 8 wherein the butanol dehydrogenase has an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:72, SEQ ID NO:75, and SEQ ID NO:91 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

21. A method for the production of 2-butanol comprising:

- 1) providing a recombinant microbial host cell comprising at least one DNA molecule encoding a polypeptide that catalyzes a substrate to product conversion selected from the group consisting of:
 - i) pyruvate to alpha-acetolactate;
 - ii) alpha-acetolactate to acetoin;
 - iii) acetoin to 2,3-butanediol;
 - iv) 2,3-butanediol to 2-butanone; and
 - v) 2-butanone to 2-butanol;

wherein the at least one DNA molecule is heterologous to said microbial host cell; and

- 2) contacting the host cell of (1) with a fermentable carbon substrate in a fermentation medium under conditions whereby 2-butanol is produced.

22. A method for the production of 2-butanone comprising:
- 1) providing a recombinant microbial host cell comprising at least one DNA molecule encoding a polypeptide that catalyzes a substrate to product conversion selected from the group consisting of:
 - i) pyruvate to alpha-acetolactate;
 - ii) alpha-acetolactate to acetoin;
 - iii) acetoin to 2,3-butanediol; and
 - iv) 2,3-butanediol to 2-butanone;wherein the at least one DNA molecule is heterologous to said microbial host cell; and
 - 2) contacting the host cell of (1) with a fermentable carbon substrate in a fermentation medium under conditions whereby 2-butanone is produced.
23. A method according to claims 21 or 22 wherein the fermentable carbon substrate is selected from the group consisting of monosaccharides, oligosaccharides, and polysaccharides.
24. A method according to claims 21 or 22 wherein the polypeptide that catalyzes a substrate to product conversion of pyruvate to alpha-acetolactate is acetolactate synthase.
25. A method according to claims 21 or 22 wherein the polypeptide that catalyzes a substrate to product conversion of alpha-acetolactate to acetoin is acetolactate decarboxylase.
26. A method according to claims 21 or 22 wherein the polypeptide that catalyzes a substrate to product conversion of acetoin to 2,3-butanediol is butanediol dehydrogenase.

27. A method according to claims 21 or 22 wherein the polypeptide that catalyzes a substrate to product conversion of 2,3-butanediol to 2-butanone is diol dehydratase or glycerol dehydratase.

28. A method according to claim 21 wherein the polypeptide that catalyzes a substrate to product conversion of 2-butanone to 2-butanol is butanol dehydrogenase.

29. A method according to claims 21 or 22 wherein the cell is selected from the group consisting of: a bacterium, a cyanobacterium, a filamentous fungus, and a yeast.

30. A method according to claim 29 wherein the cell is a member of a genus selected from the group consisting of *Clostridium*, *Zymomonas*, *Escherichia*, *Salmonella*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Alcaligenes*, *Klebsiella*, *Paenibacillus*, *Arthrobacter*, *Corynebacterium*, *Brevibacterium*, *Pichia*, *Candida*, *Hansenula* and *Saccharomyces*.

31. A method according to claim 24 wherein the acetolactate synthase has an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:77, and SEQ ID NO:79 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

32. A method according to claim 25 wherein the acetolactate decarboxylase has an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO: 81, and SEQ ID NO:83 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

33. A method according to claim 26 wherein the butanediol dehydrogenase has an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:85, SEQ ID NO:87, and SEQ ID NO:89 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

34. A method according to Claim 27 wherein the diol dehydratase or glycerol dehydratase comprise full length large, medium and small subunits that each give an E-value parameter of 0.01 or less when queried using a Profile Hidden Markov Model prepared using the large subunits of SEQ ID NOs: 8, 99, 105, 135, 138, 141, 146, and 164; the medium subunits of SEQ ID NOs: 10, 101, 107, 136, 139, 142, 148, and 165; and the small subunits of SEQ ID NOs: 12, 103, 109, 137, 140, 143, 150, and 166; each query being carried out using the hmmsearch algorithm wherein the Z parameter is set to 1 billion.

35. A method according to Claim 27 wherein the diol dehydratase or the glycerol dehydratase identified by a process comprising the steps of:

- a) generating a Profile Hidden Markov Model from the alignment of the amino acid sequences corresponding to the large, medium and small subunits of diol and glycerol dehydratase enzymes wherein;
 - i) the large subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 99, 105, 135, 138, 141, 146, and 164;
 - ii) the medium subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 101, 107, 136, 139, 142, 148, and 165; and
 - iii) the small subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 103, 109, 137, 140, 143, 150, and 166;

- b) querying at least one public database of protein sequences containing sequences of diol and glycerol dehydratases with the Profile Hidden Markov Model of (a) using the hmmsearch algorithm wherein the Z parameter is set to 1 billion and the E-value parameter is set to 0.01, to identify a first data set of diol and glycerol dehydratase amino acid sequences; and
- c) removing any partial sequences from the first data set of (b) to generate a second data set of diol and glycerol dehydratase amino acid sequences, wherein diol dehydratase and the glycerol dehydratase enzymes are identified.

36. A method according to Claim 27 wherein the diol dehydratase or glycerol dehydratase comprises a large subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 93, 99, 105, 135, 138, 141, 146, 164, 167, 170, 173, 176, 179, 182, 185, 188, 191, 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 130, 243, 254, 255, 256, 257, 258 and 259, based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

37. A method according to Claim 27 wherein the diol dehydratase or glycerol dehydratase comprises a medium subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 95, 101, 107, 136, 139, 142, 148, 165, 168, 171, 174, 177, 180, 183, 186, 189, 192, 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 244, 250, 252, 260, 261, 262, 263, 364, 265, 266, and 167 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

38. A method according to Claim 27 wherein the diol dehydratase or glycerol dehydratase comprises a small subunit comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 97, 103, 109, 137, 140, 143, 150, 166, 169, 172, 175, 178, 181, 184, 187, 190, 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 234, 236, 238, 240, 242, 245, 248, 249, 251, 253, 268, 270, 271, 272, 273, and 274, based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

39. A method according to Claim 27 wherein the diol dehydratase or glycerol dehydratase comprises fused large, medium and small subunits comprising an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 233, 235, 237, 239, 241, 246, and 247, based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

40. A method I according to Claim 27 wherein the diol dehydratase or glycerol dehydratase comprises fused large, medium and small subunits and has at least 95% identity to an amino acid sequence comprising all three of the amino acid sequences encoding large, medium and small subunits, selected from the group consisting of:

- a) SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12;
- b) SEQ ID NO:93, SEQ ID NO:95, and SEQ ID NO:97;
- c) SEQ ID NO:99, SEQ ID NO:101, and SEQ ID NO:103;
- d) SEQ ID NO:105, SEQ ID NO:107, and SEQ ID NO:109;
- e) SEQ ID NO:135, SEQ ID NO:136, and SEQ ID NO:137;
- f) SEQ ID NO:138, SEQ ID NO:139, and SEQ ID NO:140;
- g) SEQ ID NO:146, SEQ ID NO:148, and SEQ ID NO:150;
- h) SEQ ID NO:141, SEQ ID NO:142, and SEQ ID NO:143; and
- i) SEQ ID NO:164, SEQ ID NO:165, and SEQ ID NO:166;

based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

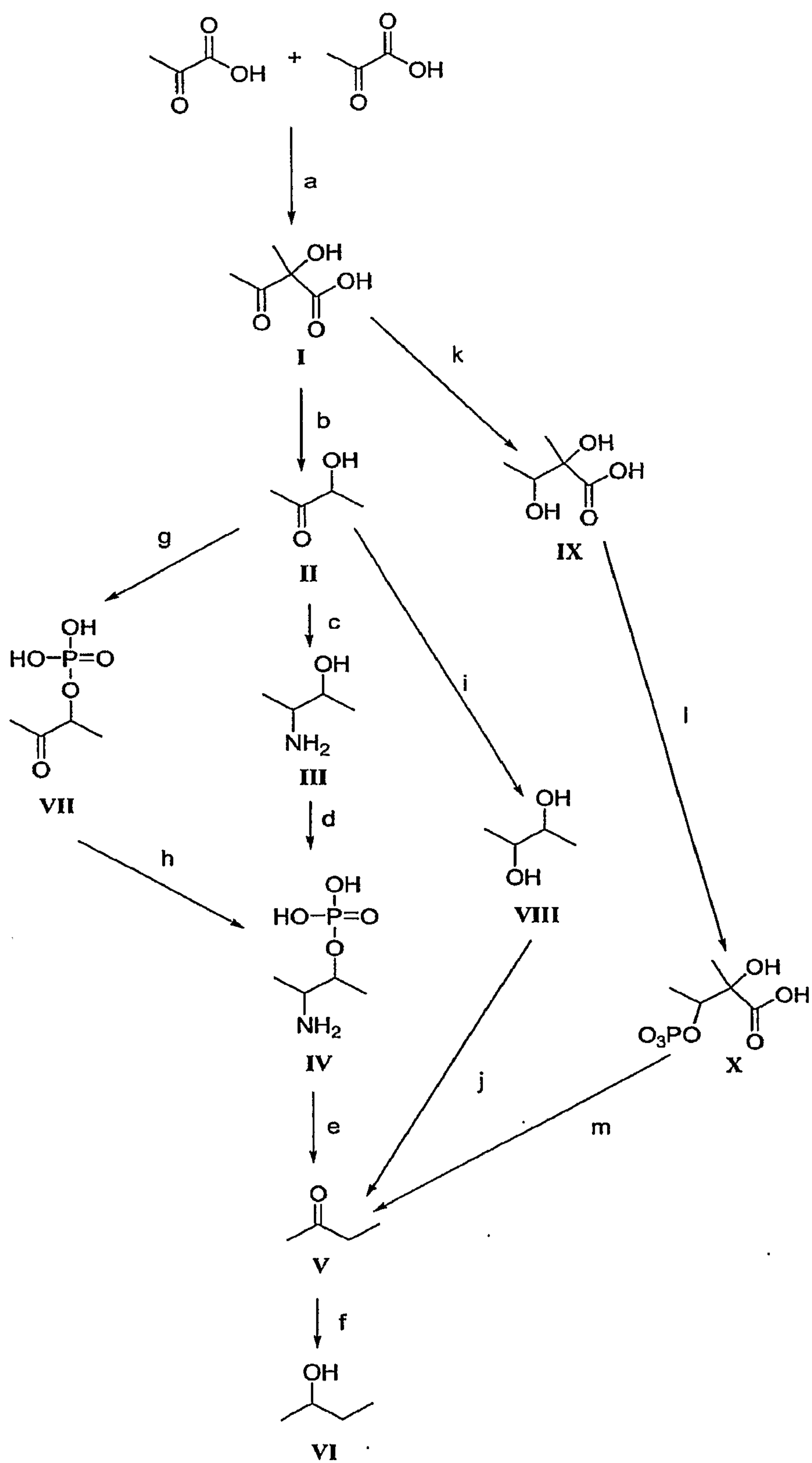
41. A method according to claim 28 wherein the butanol dehydrogenase has an amino acid sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:72, SEQ ID NO:75, and SEQ ID NO:91 based on the Clustal W method of alignment using the default parameters of GAP PENALTY=10, GAP LENGTH PENALTY=0.1, and Gonnet 250 series of protein weight matrix.

42. A 2-butanol containing fermentation product medium produced by the method of claim 21.

43. A 2-butanone containing fermentation product medium produced by the method of claim 22.

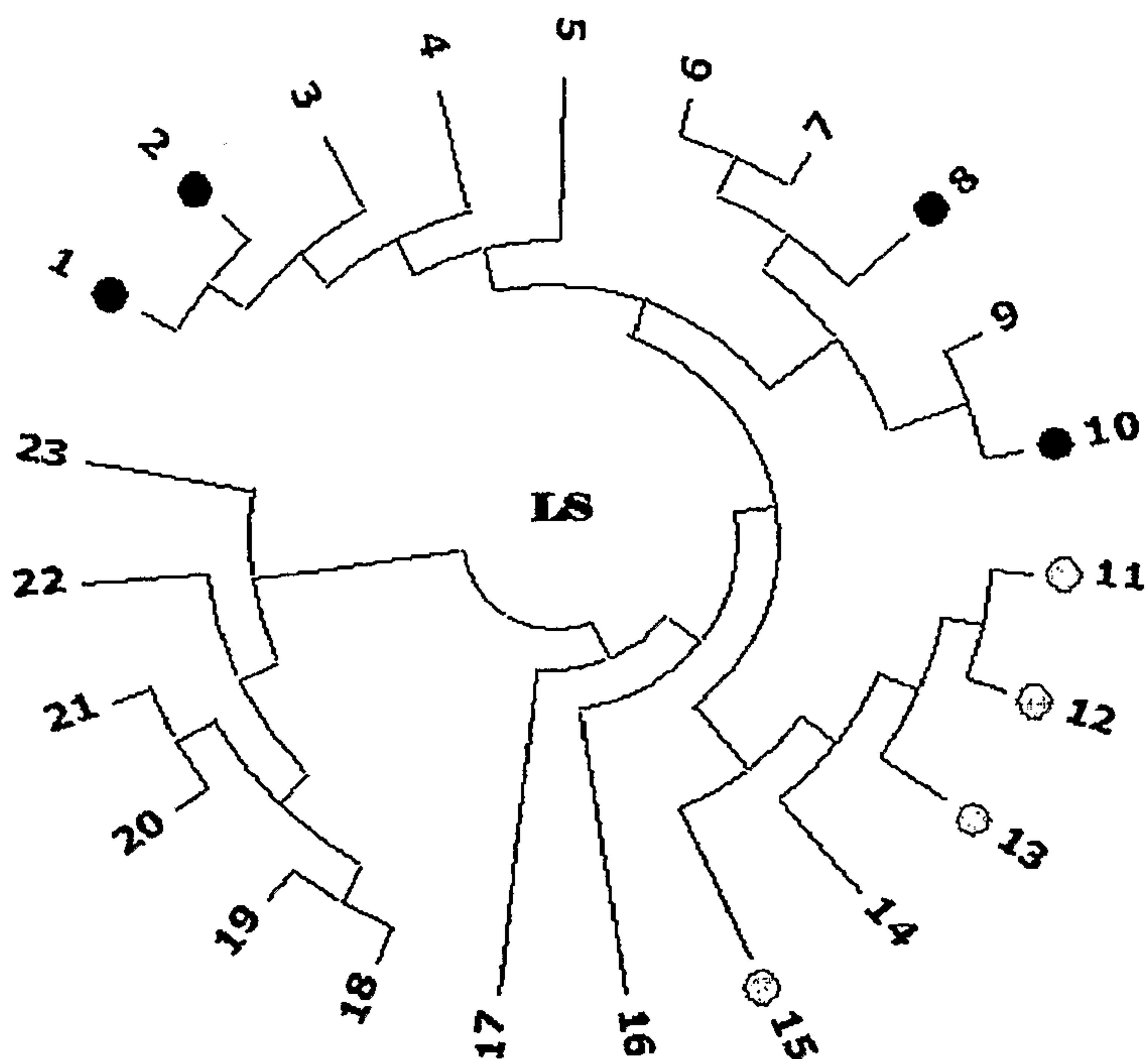
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Figure 1



2/4

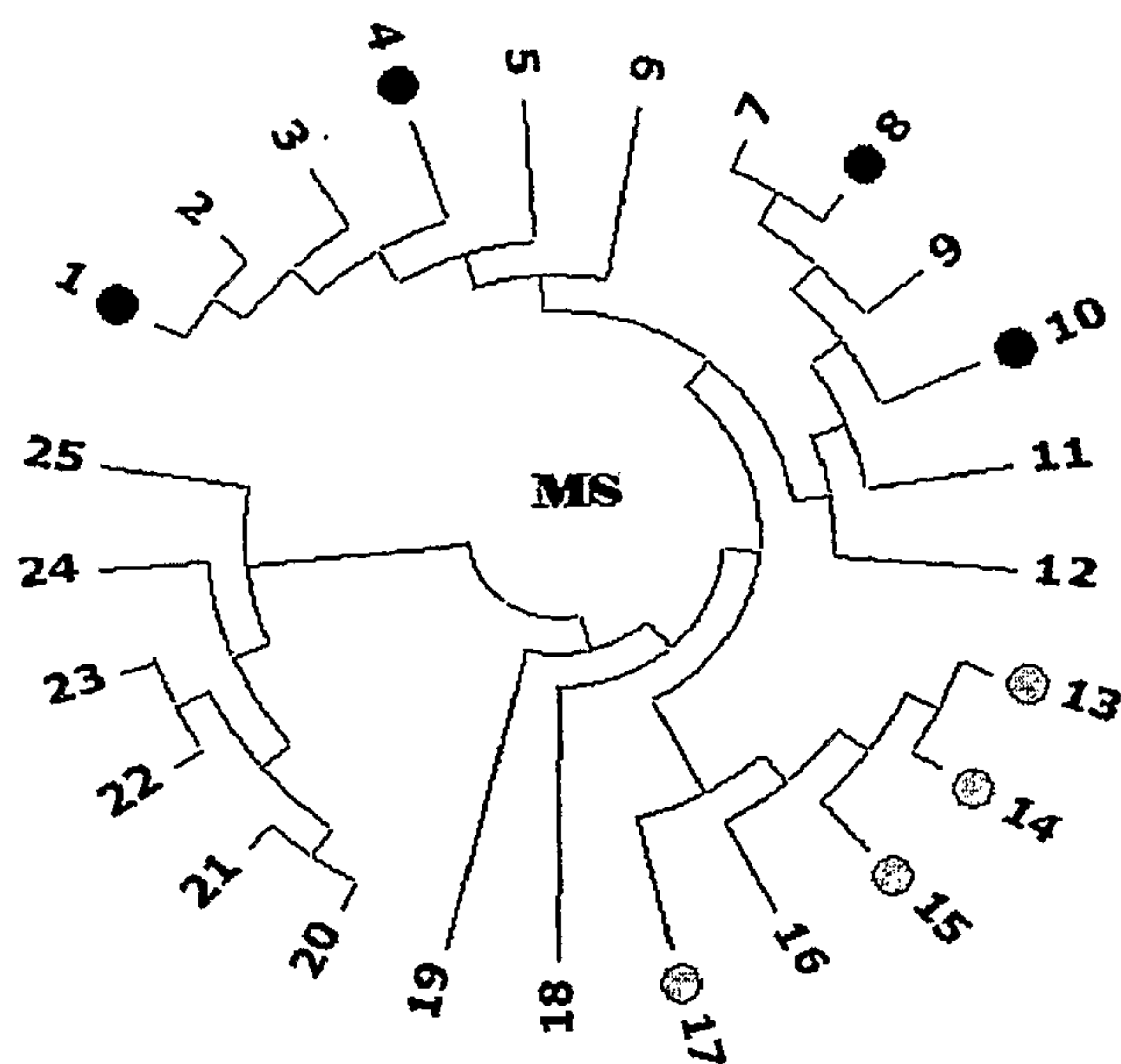
Figure 2



1	gi 4063702 DD <i>Klebsiella pneumoniae</i>
2	gi 6980836 DD <i>Klebsiella Oxytoca</i> pdb 1D
3	gi 123442946 <i>Yersinia enterocolitica</i>
4	gi 116872527 <i>Listeria welshimeri</i>
5	gi 125717397 <i>Streptococcus sanguinis</i>
6	gi 37542647 <i>Lactobacillus diolivorans</i>
7	gi 37542653 <i>Lactobacillus hilgardii</i>
8	gi 18857678 DD <i>Lactobacillus collinoides</i>
9	gi 86604356 <i>Lactobacillus reuteri</i>
10	gi 116334196 DD <i>Lactobacillus brevis</i>
11	gi 1169287 GD <i>Citrobacter freundii</i>
12	gi 24158719 GD <i>Klebsiella pneumoniae</i>
13	gi 60099613 GD <i>Escherichia blattae</i>
14	gi 18309911 <i>Clostridium perfringens</i>
15	gi 3360389 GD <i>Clostridium pasteurianum</i>
16	gi 114844379 <i>Thermoanaerobacter ethanolicus</i>
17	gi 118472362 <i>Mycobacterium smegmatis</i>
18	gi 108801917 <i>Mycobacterium</i> sp. MCS
19	gi 118472557 <i>Mycobacterium smegmatis</i> str. MC2 155
20	gi 120406505 <i>Mycobacterium vanbaalenii</i> PYR-1
21	gi 89342701 <i>Mycobacterium flavescens</i>
22	gi 118470012 <i>Mycobacterium smegmatis</i> str. MC2 155
23	gi 13475607 <i>Mesorhizobium loti</i> MAFF303099

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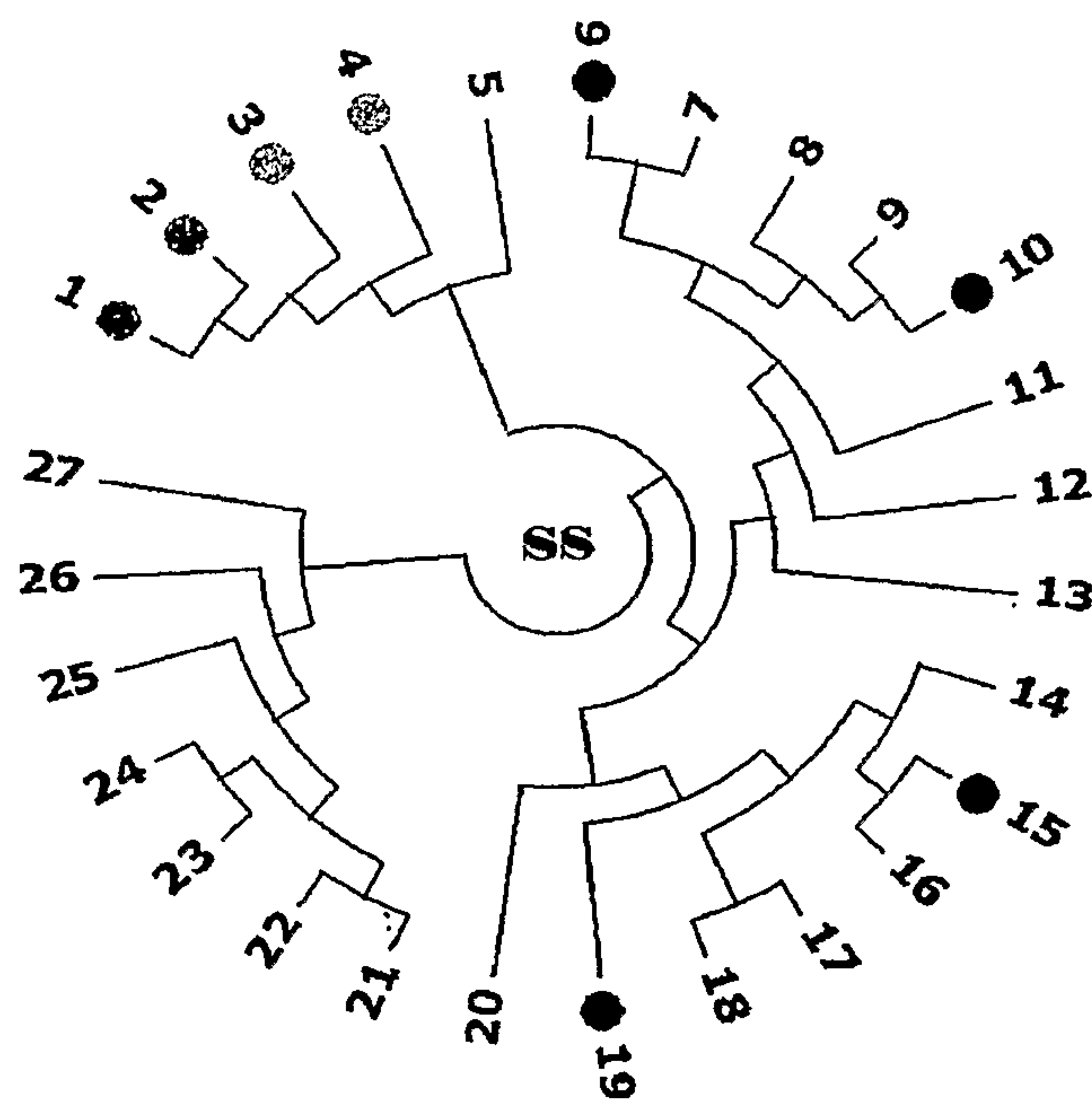
Figure 3



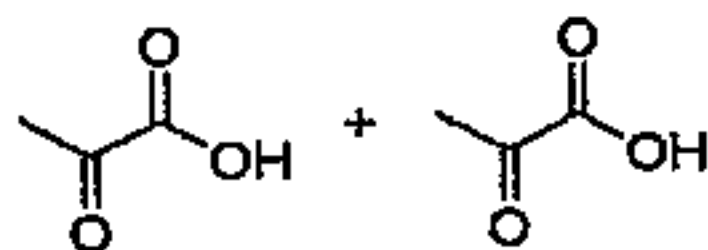
1	gi 6980837 DDm Klebsiella Oxytoca
2	gi 74312533 Shigella sonnei Ss046
3	gi 31324737 uncultured bacterium
4	gi 94470233 DDm Klebsiella pneumoniae
5	gi 77962425 Yersinia mollaretii ATCC 43969
6	gi 116872528 Listeria welshimeri
7	gi 37542648 Lactobacillus diolivorans
8	gi 18857679 DDm Lactobacillus collinoides
9	gi 37542654 Lactobacillus hilgardii
10	gi 116334195 DDm Lactobacillus brevis
11	gi 86604354 Lactobacillus reuteri
12	gi 125717398 Streptococcus sanguinis SK36
13	gi 1229154 GDm Citrobacter freundii
14	gi 24158720 GDm Klebsiella pneumoniae
15	gi 57340191 GDm Escherichia blattae
16	gi 18309912 Clostridium perfringens
17	gi 3360390 GDm Clostridium pasteurianum
18	gi 114844378 Thermoanaerobacter ethanolicus X514
19	gi 118467606 Mycobacterium smegmatis str. MC2 155
20	gi 108801917 Mycobacterium sp. MCS
21	gi 118472557 Mycobacterium smegmatis str. MC2 155
22	gi 120406505 Mycobacterium vanbaalenii PYR-1
23	gi 89342701 Mycobacterium flavescens
24	gi 118470012 Mycobacterium smegmatis str. MC2 155
25	gi 13475607 Mesorhizobium loti MAFF303099

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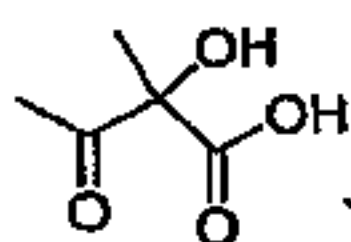
Figure 4



1	gi 1229155 GDs Citrobacter freundii
2	gi 24158721 GDs Klebsiella pneumoniae
3	gi 57340192 GDs Escherichia blatae
4	gi 3360391 GDs Clostridium pasteurianum
5	gi 110802854 Clostridium perfringens SM101
6	gi 6980838 DDs Klebsiella oxytoca
7	gi 31324742 uncultured bacterium
8	gi 74312534 Shigella sonnei Ss046
9	gi 4063704 Klebsiella pneumoniae
10	gi 4063704 DDs Klebsiella pneumoniae
11	gi 77962426 Yersinia mollaretii ATCC 43969
12	gi 116872529 Listeria welshimeri
13	gi 13475606 Mesorhizobium loti
14	gi 37542649 Lactobacillus diolivorans
15	gi 18857680 DDs Lactobacillus collinoides
16	gi 37542655 Lactobacillus hilgardii
17	gi 89090283 Lactobacillus reuteri JCM 1112
18	gi 86604352 Lactobacillus reuteri
19	gi 116334194 DDs Lactobacillus brevis
20	gi 125717399 Streptococcus sanguinis SK36
21	gi 108801916 Mycobacterium sp. MCS
22	gi 118469461 Mycobacterium smegmatis str. MC2 155
23	gi 120406504 Mycobacterium vanbaalenii PYR-1
24	gi 89342702 Mycobacterium flavescens
25	gi 118468095 Mycobacterium smegmatis str. MC2 155
26	gi 118472086 Mycobacterium smegmatis str. MC2 155
27	gi 114844377 Thermoanaerobacter ethanolicus X514

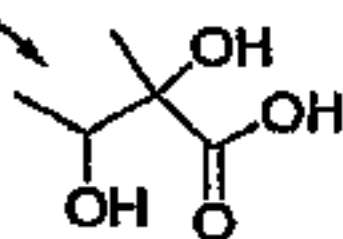


a



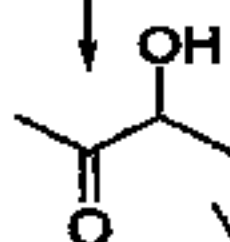
I

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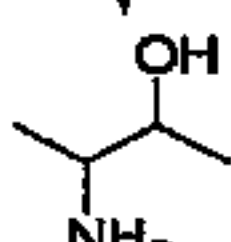
IX

b



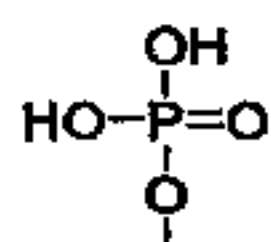
II

c



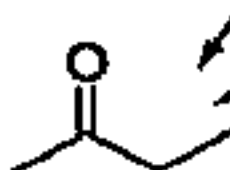
III

d



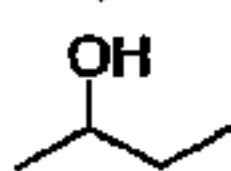
IV

e



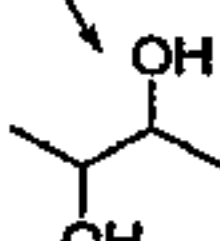
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VI

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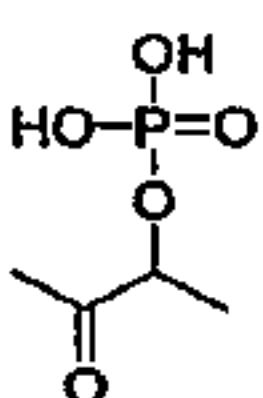
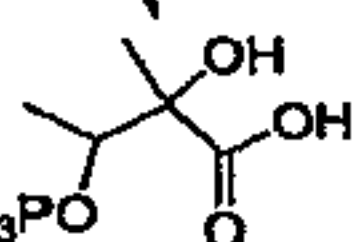
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VII

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