(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 August 2011 (25.08.2011)

PCT

(10) International Publication Number
WO 2011/103300 A2

(51) International Patent Classification:
C12N 1/19 (2006.01) *C12P 7/16* (2006.01)
C12N 15/52 (2006.01) *G01N 33/68* (2006.01)

(21) International Application Number:
PCT/US2011/025258

(22) International Filing Date:
17 February 2011 (17.02.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/305,333 17 February 2010 (17.02.2010) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

[Continued on next page]

(54) Title: IMPROVING ACTIVITY OF FE-S CLUSTER REQUIRING PROTEINS

Figure 1A

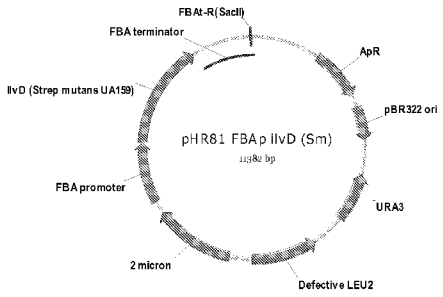
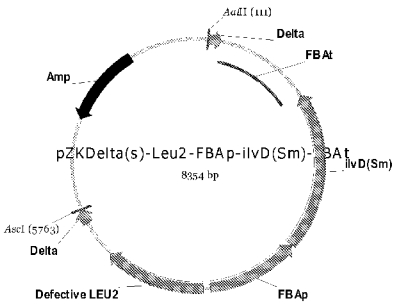


Figure 1B



(57) Abstract: The present invention is related to a recombinant host cell, in particular a yeast cell, comprising a dihydroxy-acid dehydratase polypeptide. The invention is also related to a recombinant host cell having increased specific activity of the dihydroxy-acid dehydratase polypeptide as a result of increased expression of the polypeptide, modulation of the Fe-S cluster biosynthesis of the cell, or a combination thereof. The present invention also includes methods of using the host cells, as well as, methods for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell.

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SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *with sequence listing part of description (Rule 5.2(a))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

IMPROVING ACTIVITY OF FE-S CLUSTER REQUIRING PROTEINS

Cross-Reference to Related Applications

[0001] This application claims the benefit of U.S. Provisional Appl. No. 61/305,333, filed February 17, 2010, which is incorporated by reference in its entirety.

Sequence Listing Information

[0002] The content of the electronically submitted sequence listing in ASCII text file CL4842sequencelisting.txt filed with the application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0003] This invention relates generally to the fields of microbiology and biochemistry. Specifically, the present invention is related to a recombinant host cell, in particular a yeast cell, comprising a dihydroxy-acid dehydratase polypeptide. The invention is also related to a recombinant host cell having increased specific activity of the dihydroxy-acid dehydratase polypeptide as a result of increased expression of the polypeptide, modulation of the Fe-S cluster biosynthesis activity of the cell, or a combination thereof. The present invention also includes methods of using the host cells, as well as methods for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell.

Background of the Invention

[0004] Iron-sulfur (Fe-S) clusters serve as cofactors or prosthetic groups essential for the normal function of the class of proteins that contain them. In the class of Fe-S cluster containing proteins, the Fe-S clusters have been found to play several roles. When proteins of this class are first synthesized by the cell, they lack the Fe-S clusters required for their proper function and are referred to as apoproteins. Fe-S clusters are made in a series of reactions by proteins involved in Fe-S cluster biosynthesis and are transferred to the apo-proteins to form the functional Fe-S cluster containing holoproteins.

[0005] One such protein that requires Fe-S clusters for proper function is dihydroxy-acid dehydratase (DHAD) (E.C. 4.2.1.9). DHAD catalyzes the conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate, and of 2,3-dihydroxymethylvalerate to α-ketomethylvalerate. The DHAD enzyme is part of naturally occurring biosynthetic pathways producing the branched chain amino acids, (i.e., valine, isoleucine, leucine), and pantothenic acid (vitamin B5). DHAD catalyzed conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate is also a common step in the multiple isobutanol biosynthetic pathways that are disclosed in U.S. Patent Appl. Pub. No. US 20070092957 A1, incorporated by reference herein. Disclosed therein is, *e.g.*, the engineering of recombinant microorganisms for the production of isobutanol.

[0006] High levels of DHAD activity are desired for increased production of products from biosynthetic pathways that include this enzyme activity, including, *e.g.*, enhanced microbial production of branched chain amino acids, pantothenic acid, and isobutanol. Isobutanol, in particular, is useful as a fuel additive, and its ready availability may reduce the demand for petrochemical fuels. However, since all known DHAD enzymes require a Fe-S cluster for their function, they must be expressed in a host having the genetic machinery to provide the Fe-S clusters required by these proteins. In yeast, mitochondria play an essential role in Fe-S cluster biosynthesis. If the DHAD is to be functionally expressed in yeast cytosol, a system to transport the requisite Fe-S precursor or signal from mitochondria and assemble the Fe-S cluster on the cytosolic apoprotein is required. Prior to the work of the present inventors, it was previously unknown whether yeast could provide Fe-S clusters for any DHAD located in the cytoplasm (since native yeast DHAD is located in the mitochondria) and more importantly when the DHAD is expressed at high levels in the cytoplasm

[0007] Under certain conditions the rate of synthesis of Fe-S cluster requiring apo-proteins may exceed the cell's ability to synthesize and assemble Fe-S clusters for them. Cluster-less apo-proteins that accumulate under these conditions cannot carry out their normal function. Such conditions can include 1) the expression of a heterologous Fe-S cluster requiring protein especially in high amounts, 2) the expression of a native Fe-S cluster biosynthesis protein at higher levels than normal, or 3) a state where the host cell's ability to synthesize Fe-S clusters is debilitated.

BRIEF SUMMARY OF THE INVENTION

[0008] Disclosed herein is the surprising discovery that recombinant host cells expressing a high level of a heterologous Fe-S cluster requiring protein can supply the complement of Fe-S clusters for that protein if the level(s) of at least one Fe uptake, utilization, and/or Fe-S cluster biosynthesis protein are altered.

[0009] Provided herein are recombinant host cells comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity wherein said at least one heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated. Also provided are recombinant host cells comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity wherein said at least one heterologous polynucleotide is integrated at least once in the recombinant host cell DNA. Also provided are recombinant host cells comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity, wherein said host cell comprises at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting iron metabolism or Fe-S cluster biosynthesis. Also provided are recombinant host cells comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity and at least one heterologous polynucleotide encoding a polypeptide affecting iron metabolism or Fe-S cluster biosynthesis.

[0010] In embodiments, said heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of the genes in Tables 7, 8 and 9. In embodiments, said heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of AFT1, AFT2, CCC1, FRA2, and GRX3, and combinations thereof. In embodiments, polypeptide is encoded by a polynucleotide that is constitutive mutant. In embodiments, said constitutive mutant is selected from the group consisting of AFT1 L99A, AFT1 L102A, AFT1 C291F, AFT1 C293F, and combinations thereof. In embodiments said polypeptide affecting Fe-S cluster biosynthesis is encoded by a polynucleotide comprising a high copy number plasmid or a plasmid with a copy number that can be regulated. In embodiments, said polypeptide affecting Fe-S cluster biosynthesis is encoded by a polynucleotide integrated at least once in the recombinant host cell DNA.

In embodiments, the at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of CCC1, FRA2, and GRX3, and combinations thereof. In embodiments, the at least one heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of AFT1, AFT2, their mutants, and combinations thereof.

[0011] In embodiments, said at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity is expressed in multiple copies. In embodiments, said at least one heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated. In embodiments, said at least one heterologous polynucleotide is integrated at least once in the recombinant host cell DNA. In embodiments, said Fe-S cluster biosynthesis is increased compared to a recombinant host cell having endogenous Fe-S cluster biosynthesis.

[0012] In embodiments, said host cell is a yeast host cell. In embodiments, said yeast host cell is selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Hansenula*, *Candida*, *Kluyveromyces*, *Yarrowia*, *Issatchenkia* and *Pichia*.

[0013] In embodiments, said heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in the cytosol of the host cell. In embodiments, said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of $< 10^{-5}$ wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the *Streptococcus mutans* DHAD enzyme corresponding to SEQ ID NO:168. In embodiments, said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence with at least about 90% identity to SEQ ID NO: 168 or SEQ ID NO: 232. In embodiments said polypeptide having dihydroxy-acid dehydratase activity has a specific activity selected from the group consisting of: greater than about 5-fold with respect to the control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity, greater than about 8-fold with respect to the control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity, or greater than about 10-fold with respect to the control host cell comprising at least one heterologous

polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity. In embodiments said polypeptide having dihydroxy-acid dehydratase activity has a specific activity selected from the group consisting of: greater than about 3-fold with respect to a control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity and greater than about 6-fold with respect to the control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity. In embodiments, said polypeptide having dihydroxy-acid dehydratase activity has a specific activity selected from the group consisting of: greater than about 0.25 U/mg; greater than about 0.3 U/mg; greater than about 0.5 U/mg; greater than about 1.0 U/mg; greater than about 1.5 U/mg; greater than about 2.0 U/mg; greater than about 3.0 U/mg; greater than about 4.0 U/mg; greater than about 5.0 U/mg; greater than about 6.0 U/mg; greater than about 7.0 U/mg; greater than about 8.0 U/mg; greater than about 9.0 U/mg; greater than about 10.0 U/mg; greater than about 20.0 U/mg; and greater than about 50.0 U/mg.

[0014] In embodiments said recombinant host cell produces isobutanol, and in embodiments, said recombinant host cell comprises an isobutanol biosynthetic pathway.

[0015] Also provided herein are methods of making a product comprising: providing a recombinant host cell; and contacting the recombinant host cell of with a fermentable carbon substrate in a fermentation medium under conditions wherein said product is produced;, wherein the product is selected from the group consisting of branched chain amino acids, pantothenic acid, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutanol, and combinations thereof. In embodiments, the methods further comprise optionally recovering said product. In embodiments, the methods further comprise recovering said product.

[0016] Also provided are methods of making isobutanol comprising: providing a recombinant host cell; contacting the recombinant host cell with a fermentable carbon substrate in a fermentation medium under conditions wherein isobutanol is produced. In embodiments, the methods further comprise optionally recovering said isobutanol. In embodiments, the methods further comprise recovering said isobutanol.

[0017] Also provided are methods for the conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate comprising: providing a recombinant host cell; growing the recombinant

host cell of under conditions where the 2,3-dihydroxyisovalerate is converted to α-ketoisovalerate. In embodiments, the conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate compared to a control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity is increased in an amount selected from the group consisting of: (a) at least about 5%; (b) at least about 10%; (c) at least about 15%; (d) at least about 20%; (e) at least about 25%; (f) at least about 30%; (g) at least about 35%; (h) at least about 40%; (i) at least about 45%; (j) at least about 50%; (k) at least about 60%; (l) at least about 70%; (m) at least about 80%; (n) at least about 90%; and (o) at least about 95%.

[0018] Also provided are methods for increasing the specific activity of a heterologous polypeptide having dihydroxy-acid dehydratase activity in a recombinant host cell comprising: providing a recombinant host cell; and growing the recombinant host cell of under conditions whereby the heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in functional form having a specific activity greater than the same host cell lacking said heterologous polypeptide.

[0019] Also provided are methods for increasing the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising: providing a recombinant host cell; and growing the recombinant host cell under conditions whereby the flux in the Fe-S cluster biosynthesis pathway in the host cell is increased.

[0020] Also provide are methods of increasing the activity of an Fe-S cluster requiring protein in a recombinant host cell comprising: providing a recombinant host cell comprising an Fe-S cluster requiring protein; changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis in said host cell; and growing the recombinant host cell under conditions whereby the activity of the Fe-S cluster requiring protein is increased. In embodiments, said increase in activity is an amount selected from the group consisting of: greater than about 10%; greater than about 20%; greater than about 30%; greater than about 40%; greater than about 50%; greater than about 60%; greater than about 70%; greater than about 80%; greater than about 90%; and greater than about 95%, 98%, or 99%. In embodiments, the increase in activity is in an amount selected from the group consisting of: greater than about 5-fold; greater than about 8-fold; greater than about 10-fold. In embodiments, the increase in activity is in an amount

selected from the group consisting of: greater than about 3-fold and greater than about 6-fold.

[0021] A method for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising: changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis; measuring the activity of a heterologous Fe-S cluster requiring protein; and comparing the activity of the heterologous Fe-S cluster requiring protein measured in the presence of the change in expression or activity of a polypeptide to the activity of the heterologous Fe-S cluster requiring protein measured in the absence of the change in expression or activity of a polypeptide, wherein an increase in the activity of the heterologous Fe-S cluster requiring protein indicates an increase in the flux in said Fe-S cluster biosynthesis pathway.

[0022] Provided herein are methods for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising: changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis; measuring the activity of a polypeptide having dihydroxy-acid dehydratase activity; and comparing the activity of the polypeptide having dihydroxy-acid dehydratase activity measured in the presence of the change to the activity of the polypeptide having dihydroxy-acid dehydratase activity measured in the absence of change, wherein an increase in the activity of the polypeptide having dihydroxy-acid dehydratase activity indicates an increase in the flux in said Fe-S cluster biosynthesis pathway.

[0023] In embodiments, said changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis comprises deleting, mutating, substituting, expressing, up-regulating, down-regulating, altering the cellular location, altering the state of the protein, and/or adding a cofactor. In embodiments, the Fe-S cluster requiring protein has dihydroxy-acid dehydratase activity and wherein said Fe-S cluster requiring protein having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of $< 10^{-5}$ wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the *Streptococcus mutans* DHAD enzyme corresponding to SEQ ID NO:168. In embodiments, the polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of the genes in Tables 7, 8 and 9.

[0024] Also provided are recombinant host cells comprising at least one polynucleotide encoding a polypeptide identified by the methods provided herein. In embodiments, said host cell further comprises at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity. In embodiments, said heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity is expressed in multiple copies. In embodiments, said heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated. In embodiments, said heterologous polynucleotide is integrated at least once in the recombinant host cell DNA.

[0025] In embodiments, said host cell is a yeast host cell. In embodiments, said yeast host cell is selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Hansenula*, *Candida*, *Kluyveromyces*, *Yarrowia*, *Issatchenkia,* and *Pichia*. In embodiments, said heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in the cytosol of the host cell. In embodiments, said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of $< 10^{-5}$ wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the *Streptococcus mutans* DHAD enzyme corresponding to SEQ ID NO:168. In embodiments, said recombinant host cell produces a product selected from the group consisting of branched chain amino acids, pantothenic acid, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutanol, and combinations thereof. In embodiments, recombinant host cell produces isobutanol. In embodiments, said recombinant host cell comprises an isobutanol biosynthetic pathway. In embodiments said isobutanol biosynthetic pathway comprises at least one polypeptide encoded by a polynucleotide heterologous to the host cell. In embodiments, said isobutanol biosynthetic pathway comprises at least two polypeptides encoded by polynucleotides heterologous to the host cell.

[0026] In embodiments, monomers of the polypeptides of the invention having dihydroxy-acid dehydratase activity have an Fe-S cluster loading selected from the group consisting of: (a) at least about 10%; (b) at least about 15%; (c) at least about 20%; (d) at least about 25%; (e) at least about 30%; (f) at least about 35%; (g) at least about 40%; (h)

at least about 45%; (i) at least about 50%; (j) at least about 60%; (k) at least about 70%; (l) at least about 80%; (m) at least about 90%; and (n) at least about 95%.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0027] Figure 1A depicts a vector map of a vector for overexpression of the *IlvD* gene from *S. mutans*.

[0028] Figure 1B depicts a vector map of an integration vector for overexpression of the *IlvD* gene from *S. mutans* in the chromosome.

[0029] Figure 2 depicts a vector map of a centromere vector used to clone *AFT1* or *AFT1* mutants and useful for other genes of interest.

[0030] Figure 3 depicts a UV-Vis absorbance spectrum of purified *S. mutans* DHAD.

[0031] Figure 4 depicts an EPR spectrum of purified *S. mutans* DHAD.

[0032] Figure 5 depicts a biosynthetic pathway for biosynthesis of isobutanol.

[0033] Figure 6A depicts a schematic of *Azotobacter vinelandii nif* genes.

[0034] Figure 6B depicts a schematic of additional *Azotobacter vinelandii nif* genes.

[0035] Figure 6C depicts a schematic of the equation in which NFU acts as a persulfide reductase.

[0036] Figure 7 depicts a schematic of *Helicobacter pylori nif* genes.

[0037] Figure 8 depicts a schematic of *E. coli isc* genes.

[0038] Figure 9 depicts a schematic of *E. coli suf* genes.

[0039] Figure 10 depicts a schematic of the cytosolic [2Fe-2S] biosynthesis and assembly system.

[0040] Figure 11 depicts a vector map of a vector for overexpression of the *IlvD* gene from *L. lactis*.

[0041] Table 12 is a table of the Profile HMM for dihydroxy-acid dehydratases based on enzymes with assayed function prepared as described in U.S. Patent Appl. No. 12/569,636, filed Sept. 29, 2009. Table 12 is submitted herewith electronically and is incorporated herein by reference.

DETAILED DESCRIPTION OF THE INVENTION

[0042] Described herein is a method to increase the fraction of the Fe-S cluster requiring proteins that are loaded with Fe-S clusters. Also described are recombinant host cells that express functional Fe-S cluster requiring proteins, such as DHAD enzymes, and at least one heterologous Fe uptake, utilization, or Fe-S cluster biosynthesis protein, recombinant host cells that express functional DHAD enzymes and comprise at least one deletion, mutation, and/or substitution in a native protein involved in Fe utilization or Fe-S cluster biosynthesis, or recombinant host cells comprising combinations thereof. In addition, the present invention describes a method to identify polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell. Also described is a method to identify polypeptides that alter the activity of an Fe-S cluster requiring protein.

[0043] Definitions

[0044] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions will control. Also, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications, patents and other references mentioned herein are incorporated by reference in their entireties for all purposes.

[0045] In order to further define this invention, the following terms and definitions are herein provided.

[0046] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains" or "containing," or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0047] As used herein, the term "consists of," or variations such as "consist of" or "consisting of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, but that no additional integer or group of integers may be added to the specified method, structure, or composition.

[0048] As used herein, the term "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional inclusion of any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition. *See* M.P.E.P. § 2111.03.

[0049] Also, the indefinite articles "a" and "an" preceding an element or component of the invention are intended to be nonrestrictive regarding the number of instances, *i.e.*, occurrences of the element or component. Therefore "a" or "an" should be read to include one or at least one, and the singular word form of the element or component also includes the plural unless the number is obviously meant to be singular.

[0050] 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 application.

[0051] As used herein, the term "about" modifying the quantity of an ingredient or reactant of the invention employed refers to variation in the numerical quantity that can occur, for example, through typical measuring and liquid handling procedures used for making concentrates or solutions in the real world; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of the ingredients employed to make the compositions or to carry out the methods; and the like. The term "about" also encompasses amounts that differ due to different equilibrium conditions for a composition resulting from a particular initial mixture. Whether or not modified by the term "about", the claims include equivalents to the quantities. In one embodiment, the term "about" means within 10% of the reported numerical value, preferably within 5% of the reported numerical value.

[0052] The term "isobutanol biosynthetic pathway" refers to an enzyme pathway to produce isobutanol from pyruvate.

[0053] The term "a facultative anaerobe" refers to a microorganism that can grow in both aerobic and anaerobic environments.

[0054] 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.

[0055] The term "Fe-S cluster biosynthesis" refers to biosynthesis of Fe-S clusters, including, *e.g.*, the assembly and loading of Fe-S clusters. The term "Fe-S cluster biosynthesis genes", "Fe-S cluster biosynthesis proteins" or "Fe-S cluster biosynthesis pathway" refers to those polynucleotides/genes and the encoded polypeptides that are involved in the biosynthesis of Fe-S clusters, including, *e.g.*, the assembly and loading of Fe-S clusters.

[0056] The term "Fe uptake and utilization" refers to processes which can effect Fe-S cluster biosynthesis such as Fe sensing, uptake, utilization, and homeostasis. "Fe uptake and utilization genes" refers to those polynucleotides/genes and the encoded polypeptides that are involved in Fe uptake, utilization, and homeostasis. Some of these polynucleotides/genes are contained in the "Fe Regulon" that has been described in the literature and is further described hereafter. As used herein, Fe uptake and utilization genes and Fe-S cluster biosynthesis genes can encode a polypeptide affecting Fe-S cluster biosynthesis.

[0057] The term "specific activity" as used herein is defined as the units of activity in a given amount of protein. Thus, the specific activity is not directly measured but is calculated by dividing 1) the activity in units/ml of the enzyme sample by 2) the concentration of protein in that sample, so the specific activity is expressed as units/mg. The specific activity of a sample of pure, fully active enzyme is a characteristic of that enzyme. The specific activity of a sample of a mixture of proteins is a measure of the relative fraction of protein in that sample that is composed of the active enzyme of interest. The specific activity of a polypeptide of the invention may be selected from greater than about 0.25 U/mg; greater than about 0.3 U/mg; greater than about 0.4 U/mg; greater than about 0.5 U/mg; greater than about 0.6 U/mg; greater than about 0.7 U/mg; greater than about 0.8 U/mg; greater than about 0.9 U/mg; greater than about 1.0 U/mg; greater than about 1.5 U/mg; greater than about 2.0 U/mg; greater than about 2.5 U/mg;

greater than about 3.0 U/mg; greater than about 3.5 U/mg; greater than about 4.0 U/mg; greater than about 5.5 U/mg; greater than about 5.0 U/mg; greater than about 6.0 U/mg; greater than about 6.5 U/mg; greater than about 7.0 U/mg; greater than about 7.5 U/mg; greater than about 8.0 U/mg; greater than about 8.5 U/mg; greater than about 9.0 U/mg; greater than about 9.5 U/mg; greater than about 10.0 U/mg; greater than about 20.0 U/mg; or greater than about 50.0 U/mg. In one embodiment, the specific activity of a polypeptide of the invention is greater than about 0.25 U/mg. In another embodiment, the specific activity is greater than about 1.0 U/mg. In yet another embodiment, the specific activity is greater than about 2.0 U/mg or greater than about 3.0 U/mg.

[0058] The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to a nucleic acid molecule or construct, *e.g.*, messeger RNA (mRNA) or plasmid DNA (pDNA). A polynucleotide can contain the nucleotide sequence of the full-length cDNA sequence, or a fragment thereof, including the untranslated 5' and 3' sequences and the coding sequences. The polynucleotide can be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. "Polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0059] A polynucleotide sequence may be referred to as "isolated," in which it has been removed from its native environment. For example, a heterologous polynucleotide encoding a polypeptide or polypeptide fragment having dihydroxy-acid dehydratase activity contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. An isolated polynucleotide fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

[0060] The term "gene" refers to a polynucleotide 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.

[0061] As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions of the present invention can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions.

[0062] The term "endogenous," when used in reference to a polynucleotide, a gene, or a polypeptide refers to a native polynucleotide or gene in its natural location in the genome of an organism, or for a native polypeptide, is transcribed and translated from this location in the genome.

[0063] The term "heterologous" when used in reference to a polynucleotide, a gene, or a polypeptide refers to a polynucleotide, gene, or polypeptide not normally found in the host organism. "Heterologous" also includes a native coding region, or portion thereof, that is reintroduced into the source organism in a form that is different from the corresponding native gene, *e.g.*, not in its natural location in the organism's genome. The heterologous polynucleotide or gene may be introduced into the host organism by, *e.g.*, gene transfer. A heterologous gene may include a native coding region with non-native regulatory regions that is reintroduced into the native host. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

[0064] The term "recombinant genetic expression element" refers to a nucleic acid fragment that expresses one or more specific proteins, including regulatory sequences preceding (5' non-coding sequences) and following (3' termination sequences) coding sequences for the proteins. A chimeric gene is a recombinant genetic expression element. The coding regions of an operon may form a recombinant genetic expression element, along with an operably linked promoter and termination region.

[0065] "Regulatory sequences" refers 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, enhancers, operators, repressors, transcription termination signals, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing site, effector binding site and stem-loop structure.

[0066] The term "promoter" refers to a nucleic acid 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 nucleic acid 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". "Inducible promoters," on the other hand, cause a gene to be expressed when the promoter is induced or turned on by a promoter-specific signal or molecule. 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.

[0067] 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.

[0068] The term "expression", as used herein, refers to the transcription and 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. The process includes any manifestation of the functional presence of the expressed polynucleotide, gene, or polypeptide within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression.

[0069] The term "over-expression", as used herein, refers to expression that is higher than endogenous expression of the same or related polynucleotide or gene. A heterologous polynucleotide or gene is also over-expressed if its expression is higher than that of a comparable endogenous gene, or if its expression is higher than that of the same polynucleotide or gene introduced by a means that does not overexpress the polynucleotide or gene. For example, a polynucleotide can be expressed in a host cell from a low copy number plasmid, which is present in only limited or few copies, and the same polynucleotide can be over-expressed in a host cell from a high copy number plasmid or a plasmid with a copy number that can be regulated, which is present in multiple copies. Any means can be used to over-express a polynucleotide, so long as it increases the copies of the polynucleotide in the host cell. In addition to using a high copy number plasmid, or a plasmid with a copy number that can be regulated, a polynucleotide can be over-expressed by multiple chromosomal integrations.

[0070] Expression or over-expression of a polypeptide of the invention in a recombinant host cell can be quantified according to any number of methods known to the skilled artisan and can be represented, *e.g.*, by a percent of total cell protein. The percent of total protein can be an amount selected from greater than about 0.001% of total cell protein; greater than about 0.01% of total cell protein; greater than about 0.1% of total cell protein; greater than about 0.5% of total cell protein; greater than about 1.0% of total cell protein; greater than about 2.0% of total cell protein; greater than about 3% of total cell protein; greater than about 4.0% of total cell protein; greater than about 5% of total cell protein; greater than about 6.0% of total cell protein; greater than about 7.0% of total cell protein; greater than about 8.0% of total cell protein; greater than about 9.0% of total cell protein; greater than about 10% of total cell protein; or greater than about 20% of total

cell protein. In one embodiment, the amount of polypeptide expressed is greater that about 0.5% of total cell protein. In another embodiment, the amount of polypeptide expressed is greater than about 1.0% of total cell protein or greater than about 2.0% of total cell protein.

[0071] As used herein the term "transformation" refers to the transfer of a nucleic acid fragment into a host organism, resulting in genetically stable inheritance with or without selections. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms.

[0072] The terms "plasmid" and "vector" as used herein, refer to an extra chromosomal element often carrying genes which are not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

[0073] As used herein the term "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 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.

[0074] 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. Such optimization includes replacing at least one, or more than one, or a significant number, of codons with one or more codons that are more frequently used in the genes of that organism.

[0075] Deviations in the nucleotide sequence that comprise the codons encoding the amino acids of any polypeptide chain allow for variations in the sequence coding for the gene. Since each codon consists of three nucleotides, and the nucleotides comprising DNA are restricted to four specific bases, there are 64 possible combinations of nucleotides, 61 of which encode amino acids (the remaining three codons encode signals ending translation). The "genetic code" which shows which codons encode which amino acids is reproduced herein as Table 1. As a result, many amino acids are designated by more than one codon. For example, the amino acids alanine and proline are coded for by four triplets, serine and arginine by six, whereas tryptophan and methionine are coded by just one triplet. This degeneracy allows for DNA base composition to vary over a wide range without altering the amino acid sequence of the proteins encoded by the DNA.

Table 1. The Standard Genetic Code

	T	**C**	**A**	**G**
T	TTT Phe (F) TTC " TTA Leu (L) TTG "	TCT Ser (S) TCC " TCA " TCG "	TAT Tyr (Y) TAC " TAA **Stop** TAG **Stop**	TGT Cys (C) TGC TGA **Stop** TGG Trp (W)
C	CTT Leu (L) CTC " CTA " CTG "	CCT Pro (P) CCC " CCA " CCG "	CAT His (H) CAC " CAA Gln (Q) CAG "	CGT Arg (R) CGC " CGA " CGG "
A	ATT Ile (I) ATC " ATA " **ATG** Met (M)	ACT Thr (T) ACC " ACA " ACG "	AAT Asn (N) AAC " AAA Lys (K) AAG "	AGT Ser (S) AGC " AGA Arg (R) AGG "
G	GTT Val (V) GTC " GTA " GTG "	GCT Ala (A) GCC " GCA " GCG "	GAT Asp (D) GAC " GAA Glu (E) GAG "	GGT Gly (G) GGC " GGA " GGG "

[0076] Many organisms display a bias for use of particular codons to code for insertion of a particular amino acid in a growing peptide chain. Codon preference, or codon bias, differences in codon usage between organisms, is afforded by degeneracy of the genetic

code, and is well documented among many organisms. Codon bias often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, *inter alia*, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization.

[0077] Given the large number of gene sequences available for a wide variety of animal, plant and microbial species, it is possible to calculate the relative frequencies of codon usage. Codon usage tables are readily available, for example, at the "Codon Usage Database" available at http://www.kazusa.or.jp/codon/ (visited March 20, 2008), and these tables can be adapted in a number of ways. *See* Nakamura, Y., *et al. Nucl. Acids Res. 28*:292 (2000). Codon usage tables for yeast, calculated from GenBank Release 128.0 [15 February 2002], are reproduced below as Table 2. This table uses mRNA nomenclature, and so instead of thymine (T) which is found in DNA, the tables use uracil (U) which is found in RNA. Table 2 has been adapted so that frequencies are calculated for each amino acid, rather than for all 64 codons.

Table 2. Codon Usage Table for *Saccharomyces cerevisiae* Genes

Amino Acid	Codon	Number	Frequency per thousand
Phe	UUU	170666	26.1
Phe	UUC	120510	18.4
Leu	UUA	170884	26.2
Leu	UUG	177573	27.2
Leu	CUU	80076	12.3
Leu	CUC	35545	5.4
Leu	CUA	87619	13.4
Leu	CUG	68494	10.5
Ile	AUU	196893	30.1
Ile	AUC	112176	17.2
Ile	AUA	116254	17.8
Met	AUG	136805	20.9
Val	GUU	144243	22.1

Amino Acid	Codon	Number	Frequency per thousand
Val	GUC	76947	11.8
Val	GUA	76927	11.8
Val	GUG	70337	10.8
Ser	UCU	153557	23.5
Ser	UCC	92923	14.2
Ser	UCA	122028	18.7
Ser	UCG	55951	8.6
Ser	AGU	92466	14.2
Ser	AGC	63726	9.8
Pro	CCU	88263	13.5
Pro	CCC	44309	6.8
Pro	CCA	119641	18.3
Pro	CCG	34597	5.3
Thr	ACU	132522	20.3
Thr	ACC	83207	12.7
Thr	ACA	116084	17.8
Thr	ACG	52045	8.0
Ala	GCU	138358	21.2
Ala	GCC	82357	12.6
Ala	GCA	105910	16.2
Ala	GCG	40358	6.2
Tyr	UAU	122728	18.8
Tyr	UAC	96596	14.8
His	CAU	89007	13.6
His	CAC	50785	7.8
Gln	CAA	178251	27.3
Gln	CAG	79121	12.1
Asn	AAU	233124	35.7
Asn	AAC	162199	24.8
Lys	AAA	273618	41.9
Lys	AAG	201361	30.8
Asp	GAU	245641	37.6
Asp	GAC	132048	20.2

Amino Acid	Codon	Number	Frequency per thousand
Glu	GAA	297944	45.6
Glu	GAG	125717	19.2
Cys	UGU	52903	8.1
Cys	UGC	31095	4.8
Trp	UGG	67789	10.4
Arg	CGU	41791	6.4
Arg	CGC	16993	2.6
Arg	CGA	19562	3.0
Arg	CGG	11351	1.7
Arg	AGA	139081	21.3
Arg	AGG	60289	9.2
Gly	GGU	156109	23.9
Gly	GGC	63903	9.8
Gly	GGA	71216	10.9
Gly	GGG	39359	6.0
Stop	UAA	6913	1.1
Stop	UAG	3312	0.5
Stop	UGA	4447	0.7

[0078] By utilizing this or similar tables, one of ordinary skill in the art can apply the frequencies to any given polypeptide sequence, and produce a nucleic acid fragment of a codon-optimized coding region which encodes the polypeptide, but which uses codons optimal for a given species.

[0079] Randomly assigning codons at an optimized frequency to encode a given polypeptide sequence, can be done manually by calculating codon frequencies for each amino acid, and then assigning the codons to the polypeptide sequence randomly. Additionally, various algorithms and computer software programs are readily available to those of ordinary skill in the art. For example, the "EditSeq" function in the Lasergene Package, available from DNAstar, Inc., Madison, WI, the backtranslation function in the VectorNTI Suite, available from InforMax, Inc., Bethesda, MD, and the "backtranslate" function in the GCG-Wisconsin Package, available from Accelrys, Inc., San Diego, CA. In addition, various resources are publicly available to codon-optimize coding region sequences, *e.g.*, the "backtranslation" function at

http://www.entelechon.com/bioinformatics/backtranslation.php?lang=eng (visited April 15, 2008) and the "backtranseq" function available at http://bioinfo.pbi.nrc.ca:8090/EMBOSS/index.html (visited July 9, 2002). Constructing a rudimentary algorithm to assign codons based on a given frequency can also easily be accomplished with basic mathematical functions by one of ordinary skill in the art.

[0080] Codon-optimized coding regions can be designed by various methods known to those skilled in the art including software packages such as "synthetic gene designer" (http://phenotype.biosci.umbc.edu/codon/sgd/index.php).

[0081] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein, " "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

[0082] By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for purposed of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0083] As used herein, the term "variant" refers to a polypeptide differing from a specifically recited polypeptide of the invention, such as DHAD, by amino acid insertions, deletions, mutations, and substitutions, created using, *e.g.*, recombinant DNA techniques, such as mutagenesis. Guidance in determining which amino acid residues may be replaced, added, or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous

polypeptides, *e.g.*, yeast or bacterial, and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequences.

[0084] Alternatively, recombinant polynucleotide variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector for expression. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide. For example, mutations can be used to reduce or eliminate expression of a target protein and include, but are not limited to, deletion of the entire gene or a portion of the gene, inserting a DNA fragment into the gene (in either the promoter or coding region) so that the protein is not expressed or expressed at lower levels, introducing a mutation into the coding region which adds a stop codon or frame shift such that a functional protein is not expressed, and introducing one or more mutations into the coding region to alter amino acids so that a non-functional or a less enzymatically active protein is expressed.

[0085] Amino acid "substitutions" may be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, *i.e.*, conservative amino acid replacements, or they may be the result of replacing one amino acid with an amino acid having different structural and/or chemical properties, *i.e.*, non-conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Alternatively, "non-conservative" amino acid substitutions may be made by selecting the differences in polarity, charge, solubility, hydrophobicity, hydrophilicity, or the amphipathic nature of any of these amino acids. "Insertions" or "deletions" may be within the range of variation as structurally or

functionally tolerated by the recombinant proteins. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

[0086] A "substantial portion" of an amino acid or nucleotide sequence is that portion comprising enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to putatively identify that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Altschul, S. F., *et al.*, *J. Mol. Biol.*, 215:403-410 (1993)). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (*e.g.*, Southern hybridization) and isolation (*e.g.*, *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to specifically identify and/or isolate a nucleic acid fragment comprising the sequence. The instant specification teaches the complete amino acid and nucleotide sequence encoding particular proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

[0087] The term "complementary" is used to describe the relationship between nucleotide bases that are capable of hybridizing to one another. For example, with respect to DNA, adenine is complementary to thymine and cytosine is complementary to guanine, and with respect to RNA, adenine is complementary to uracil and cytosine is complementary to guanine.

[0088] The term "percent identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in: 1.) Computational Molecular Biology (Lesk, A. M., Ed.) Oxford University: NY (1988); 2.) Biocomputing: Informatics and Genome Projects (Smith, D. W., Ed.) Academic: NY (1993); 3.) Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., Eds.) Humania: NJ (1994); 4.) Sequence Analysis in Molecular Biology (von Heinje, G., Ed.) Academic (1987); and 5.) Sequence Analysis Primer (Gribskov, M. and Devereux, J., Eds.) Stockton: NY (1991).

[0089] Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the MegAlign™ program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignments of the sequences is performed using the "Clustal method of alignment" which encompasses several varieties of the algorithm including the "Clustal V method of alignment" corresponding to the alignment method labeled Clustal V (described by Higgins and Sharp, *CABIOS*. 5:151-153 (1989); Higgins, D.G. *et al.*, *Comput. Appl. Biosci.,* 8:189-191 (1992)) and found in the MegAlign™ program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.). For multiple alignments, the default values correspond to GAP PENALTY=10 and GAP LENGTH PENALTY=10. Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. After alignment of the sequences using the Clustal V program, it is possible to obtain a "percent identity" by viewing the "sequence distances" table in the same program. Additionally the "Clustal W method of alignment" is available and corresponds to the alignment method labeled Clustal W (described by Higgins and Sharp, *CABIOS*. 5:151-153 (1989); Higgins, D.G. *et*

al., *Comput. Appl. Biosci.* 8:189-191(1992)) and found in the MegAlign™ v6.1 program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.). Default parameters for multiple alignment (GAP PENALTY=10, GAP LENGTH PENALTY=0.2, Delay Divergen Seqs(%)=30, DNA Transition Weight=0.5, Protein Weight Matrix=Gonnet Series, DNA Weight Matrix=IUB). After alignment of the sequences using the Clustal W program, it is possible to obtain a "percent identity" by viewing the "sequence distances" table in the same program.

[0090] 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, or in describing the corresponding polynucleotides. Useful examples of percent identities include, but are not limited to: 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or any integer percentage from 55% to 100% may be useful in describing the present invention, such as 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 polynucleotide fragments not only have the above homologies but typically comprise a polynucleotide having at least 50 nucleotides, at least 100 nucleotides, at least 150 nucleotides, at least 200 nucleotides, or at least 250 nucleotides. Further, suitable polynucleotide fragments having the above homologies encode a polypeptide having at least 50 amino acids, at least 100 amino acids, at least 150 amino acids, at least 200 amino acids, or at least 250 amino acids.

[0091] 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.

[0092] Standard recombinant DNA and molecular cloning techniques used herein 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 (1989) (hereinafter "Maniatis"); and by Silhavy, T. J., Bennan, 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).

[0093] The Functions of Fe-S Cluster-Requiring Proteins

[0094] The functions of proteins that contain Fe-S clusters are diverse. One of the more complete efforts to classify these functions is given in the following table which is adapted from Johnson, D.C., et al., *Structure, function, and formation of biological iron-sulfur clusters*. Annu. Rev. Biochem., 2005. **74**: p. 247-281.

Table 3. Functions of Biological [Fe-S] clusters[a].

Function	Examples	Cluster type
Electron transfer	Ferredoxins; redox enzymes	[2Fe-2S]; [3Fe-4S]; [4Fe-4S]
Coupled electron/proton transfer	Rieske protein	[2Fe-2S]
	Nitrogenase	[8Fe-7S]
Substrate binding and activation	(de)Hydratases	[4Fe-4S], [2Fe-2S]
	Radical SAM enzymes	[4Fe-4S]
	Acetyl-CoA synthase	Ni-Ni-[4Fe-4S], [Ni-4Fe-5S]
	Sulfite reductase	[4Fe-4S]-siroheme
Fe or cluster storage	Ferredoxins	[4Fe-4S]
	Polyferredoxins	[4Fe-4S]
Structural	Endonuclease III	[4Fe-4S]
	MutY	[4Fe-4S]
Regulation of gene expression	SoxR	[2Fe-2S]
	FNR	[4Fe-4S]/[2Fe-2S]
	IRP	[4Fe-4S]
	IscR	[2Fe-2S]
Regulation of enzyme activity	Glutamine PRPP amidotransferase	[4Fe-4S]
	Ferrochelatase	[2Fe-2S]
Disulfide reduction	Ferredoxin:thioredoxin reductase	[4Fe-4S]
	Heterodisulfide reductase	[4Fe-4S]
Sulfur donor	Biotin synthase	[2Fe-2S]

[a]Abbreviations used are SAM, S-adenosylmethionine; acetyl-CoA, acetyl coenzymeA; FNR, fumarate and nitrate reduction; IRP, iron-regulatory protein; IscR, iron-sulfur cluster assembly regulatory protein; PRPP, phosphoribosylpyrophosphate.

[0095] It is believed that an increase in the supply and the efficiency of loading Fe-S clusters into one or more of the members of the above classes will have commercial and/or medical benefits. Of the many possibilities that will be appreciated by the skilled artisan, three examples are given. 1) When an Fe-S cluster containing enzyme is used in a pathway to a fermentation product and needs to be expressed at high levels to maintain a high flux in the pathway to the product (e.g., dihydroxy-acid dehydratase in the pathway to isobutanol). 2) When an Fe-S cluster containing enzyme is used in a pathway to a fermentation product and the Fe-S cluster undergoes turnover during the catalysis (e.g., biotin synthase in the commercial fermentation of glucose to biotin). 3) In a diseased state such that the normal concentration of an Fe-S cluster containing protein important for good health is low (e.g., in cases of Friedreich's ataxia).

[0096] DHAD and DHAD Assays

[0097] DHAD is an Fe-S cluster requiring protein of the dehydratase (more properly hydro-lyase) class. A gene encoding a DHAD enzyme can be used to provide expression of DHAD activity in a recombinant host cell. DHAD catalyzes the conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate and of 2,3-dihydroxymethylvalerate to α-ketomethylvalerate and is classified as E.C. 4.2.1.9. Coding sequences for DHADs that are suitable for use in a recombinant host cell can be derived from bacterial, fungal, or plant sources. DHADs that may be used may have a [4Fe-4S] cluster or a [2Fe-2S]. Tables 4a, 4b, 5, and 6 list SEQ ID NOs for coding regions and proteins of representative DHADs that may be used in the present invention. Proteins with at least about 95% identity to certain listed sequences have been omitted for simplification, but it is understood that proteins, including those omitted for simplification, with at least about 95% sequence identity to any of the proteins listed in Tables 4a, 4b, 5, and 6 and having DHAD activity may be used as disclosed herein. Additional DHAD proteins and their encoding sequences may be identified by BLAST searching of public databases, as well known to one skilled in the art. Typically BLAST (described above) searching of publicly available databases with known DHAD sequences, such as those provided herein, is used to identify DHADs and their encoding sequences that may be expressed in the present cells. For example, DHAD proteins having amino acid sequence identities of at least about 80-85%, at least about 85-90%, at least about 90-95%, or at least about 98% sequence identity to any of the DHAD proteins of Table 3 may be expressed in the present cells. Identities are 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.

Table 4a. SEQ ID NOs of Representative Bacterial [2Fe-2S] DHAD Proteins and Encoding Sequences.

Organism of derivation	SEQ ID NO: Nucleic acid	SEQ ID NO: Peptide
Mycobacterium sp. MCS	1	2
Mycobacterium gilvum PYR-GCK	3	4
Mycobacterium smegmatis str. MC2 155	5	6
Mycobacterium vanbaalenii PYR-1	7	8
Nocardia farcinica IFM 10152	9	10

Rhodococcus sp. RHA1	11	12
Mycobacterium ulcerans Agy99	13	14
Mycobacterium avium subsp. paratuberculosis K-10	15	16
Mycobacterium tuberculosis H37Ra	17	18
Mycobacterium leprae TN *	19	20
Kineococcus radiotolerans SRS30216	21	22
Janibacter sp. HTCC2649	23	24
Nocardioides sp. JS614	25	26
Renibacterium salmoninarum ATCC 33209	27	28
Arthrobacter aurescens TC1	29	30
Leifsonia xyli subsp. xyli str. CTCB07	31	32
marine actinobacterium PHSC20C1	33	34
Clavibacter michiganensis subsp. michiganensis NCPPB 382	35	36
Saccharopolyspora erythraea NRRL 2338	37	38
Acidothermus cellulolyticus 11B	39	40
Corynebacterium efficiens YS-314	41	42
Brevibacterium linens BL2	43	44
Tropheryma whipplei TW08/27	45	46
Methylobacterium extorquens PA1	47	48
Methylobacterium nodulans ORS 2060	49	50
Rhodopseudomonas palustris BisB5	51	52
Rhodopseudomonas palustris BisB18	53	54
Bradyrhizobium sp. ORS278	55	56
Bradyrhizobium japonicum USDA 110	57	58
Fulvimarina pelagi HTCC2506	59	60
Aurantimonas sp. SI85-9A1	61	62
Hoeflea phototrophica DFL-43	63	64
Mesorhizobium loti MAFF303099	65	66
Mesorhizobium sp. BNC1	67	68
Parvibaculum lavamentivorans DS-1	69	70
Loktanella vestfoldensis SKA53	71	72
Roseobacter sp. CCS2	73	74
Dinoroseobacter shibae DFL 12	75	76
Roseovarius nubinhibens ISM	77	78
Sagittula stellata E-37	79	80
Roseobacter sp. AzwK-3b	81	82
Roseovarius sp. TM1035	83	84

Oceanicola batsensis HTCC2597	85	86
Oceanicola granulosus HTCC2516	87	88
Rhodobacterales bacterium HTCC2150	89	90
Paracoccus denitrificans PD1222	91	92
Oceanibulbus indolifex HEL-45	93	94
Sulfitobacter sp. EE-36	95	96
Roseobacter denitrificans OCh 114	97	98
Jannaschia sp. CCS1	99	100
Caulobacter sp. K31	101	102
Candidatus Pelagibacter ubique HTCC1062	103	104
Erythrobacter litoralis HTCC2594	105	106
Erythrobacter sp. NAP1	107	108
Comamonas testosterone KF-1	109	110
Sphingomonas wittichii RW1	111	112
Burkholderia xenovorans LB400	113	114
Burkholderia phytofirmans PsJN	115	116
Bordetella petrii DSM 12804	117	118
Bordetella bronchiseptica RB50	119	120
Bradyrhizobium sp. ORS278	121	122
Bradyrhizobium sp. BTAi1	123	124
Bradhyrhizobium japonicum	125	126
Sphingomonas wittichii RW1	127	128
Rhodobacterales bacterium HTCC2654	129	130
Solibacter usitatus Ellin6076	131	132
Roseiflexus sp. RS-1	133	134
Rubrobacter xylanophilus DSM 9941	135	136
Salinispora tropica CNB-440	137	138
Acidobacteria bacterium Ellin345	139	140
Thermus thermophilus HB27	141	142
Maricaulis maris MCS10	143	144
Parvularcula bermudensis HTCC2503	145	146
Oceanicaulis alexandrii HTCC2633	147	148
Plesiocystis pacifica SIR-1	149	150
Bacillus sp. NRRL B-14911	151	152
Oceanobacillus iheyensis HTE831	153	154
Staphylococcus saprophyticus subsp. saprophyticus ATCC 15305	155	156
Bacillus selenitireducens MLS10	157	158

Streptococcus pneumoniae SP6-BS73	159	160
Streptococcus sanguinis SK36	161	162
Streptococcus thermophilus LMG 18311	163	164
Streptococcus suis 89/1591	165	166
Streptococcus mutans UA159	167	168
Leptospira borgpetersenii serovar Hardjo-bovis L550	169	170
Candidatus Vesicomyosocius okutanii HA	171	172
Candidatus Ruthia magnifica str. Cm (*Calyptogena magnifica*)	173	174
Methylococcus capsulatus str. Bath	175	176
uncultured marine bacterium EB80_02D08	177	178
uncultured marine gamma proteobacterium EBAC31A08	179	180
uncultured marine gamma proteobacterium EBAC20E09	181	182
uncultured gamma proteobacterium eBACHOT4E07	183	184
Alcanivorax borkumensis SK2	185	186
Chromohalobacter salexigens DSM 3043	187	188
Marinobacter algicola DG893	189	190
Marinobacter aquaeolei VT8	191	192
Marinobacter sp. ELB17	193	194
Pseudoalteromonas haloplanktis TAC125	195	196
Acinetobacter sp. ADP1	197	198
Opitutaceae bacterium TAV2	199	200
Flavobacterium sp. MED217	201	202
Cellulophaga sp. MED134	203	204
Kordia algicida OT-1	205	206
Flavobacteriales bacterium ALC-1	207	208
Psychroflexus torquis ATCC 700755	209	210
Flavobacteriales bacterium HTCC2170	211	212
unidentified eubacterium SCB49	213	214
Gramella forsetii KT0803	215	216
Robiginitalea biformata HTCC2501	217	218
Tenacibaculum sp. MED152	219	220
Polaribacter irgensii 23-P	221	222
Pedobacter sp. BAL39	223	224
Flavobacteria bacterium BAL38	225	226
Flavobacterium psychrophilum JIP02/86	227	228

Flavobacterium johnsoniae UW101	229	230
Lactococcus lactis subsp. cremoris SK11	231	232
Psychromonas ingrahamii 37	233	234
Microscilla marina ATCC 23134	235	236
Cytophaga hutchinsonii ATCC 33406	237	238
Rhodopirellula baltica SH 1	239	240
Blastopirellula marina DSM 3645	241	242
Planctomyces maris DSM 8797	243	244
Algoriphagus sp. PR1	245	246
Candidatus Sulcia muelleri str. Hc (*Homalodisca coagulata*)	247	248
Candidatus Carsonella ruddii PV	249	250
Synechococcus sp. RS9916	251	252
Synechococcus sp. WH 7803	253	254
Synechococcus sp. CC9311	255	256
Synechococcus sp. CC9605	257	258
Synechococcus sp. WH 8102	259	260
Synechococcus sp. BL107	261	262
Synechococcus sp. RCC307	263	264
Synechococcus sp. RS9917	265	266
Synechococcus sp. WH 5701	267	268
Prochlorococcus marinus str. MIT 9313	269	270
Prochlorococcus marinus str. NATL2A	271	272
Prochlorococcus marinus str. MIT 9215	273	274
Prochlorococcus marinus str. AS9601	275	276
Prochlorococcus marinus str. MIT 9515	277	278
Prochlorococcus marinus subsp. pastoris str. CCMP1986	279	280
Prochlorococcus marinus str. MIT 9211	281	282
Prochlorococcus marinus subsp. marinus str. CCMP1375	283	284
Nodularia spumigena CCY9414	285	286
Nostoc punctiforme PCC 73102	287	288
Nostoc sp. PCC 7120	289	290
Trichodesmium erythraeum IMS101	291	292
Acaryochloris marina MBIC11017	293	294
Lyngbya sp. PCC 8106	295	296

Synechocystis sp. PCC 6803	297	298
Cyanothece sp. CCY0110	299	300
Thermosynechococcus elongatus BP-1	301	302
Synechococcus sp. JA-2-3B'a(2-13)	303	304
Gloeobacter violaceus PCC 7421	305	306
Nitrosomonas eutropha C91	307	308
Nitrosomonas europaea ATCC 19718	309	310
Nitrosospira multiformis ATCC 25196	311	312
Chloroflexus aggregans DSM 9485	313	314
Leptospirillum sp. Group II UBA	315	316
Leptospirillum sp. Group II UBA	317	318
Halorhodospira halophila SL1	319	320
Nitrococcus mobilis Nb-231	321	322
Alkalilimnicola ehrlichei MLHE-1	323	324
Deinococcus geothermalis DSM 11300	325	326
Polynucleobacter sp. QLW-P1DMWA-1	327	328
Polynucleobacter necessarius STIR1	329	330
Azoarcus sp. EbN1	331	332
Burkholderia phymatum STM815	333	334
Burkholderia xenovorans LB400	335	336
Burkholderia multivorans ATCC 17616	337	338
Burkholderia cenocepacia PC184	339	340
Burkholderia mallei GB8 horse 4	341	342
Ralstonia eutropha JMP134	343	344
Ralstonia metallidurans CH34	345	346
Ralstonia solanacearum UW551	347	348
Ralstonia pickettii 12J	349	350
Limnobacter sp. MED105	351	352
Herminiimonas arsenicoxydans	353	354
Bordetella parapertussis	355	356
Bordetella petrii DSM 12804	357	358
Polaromonas sp. JS666	359	360
Polaromonas naphthalenivorans CJ2	361	362
Rhodoferax ferrireducens T118	363	364
Verminephrobacter eiseniae EF01-2	365	366
Acidovorax sp. JS42	367	368
Delftia acidovorans SPH-1	369	370
Methylibium petroleiphilum PM1	371	372

gamma proteobacterium KT 71	373	374
Tremblaya princes	375	376
Blastopirellula marina DSM 3645	377	378
Planctomyces maris DSM 8797	379	380
Microcystis aeruginosa PCC 7806	381	382
Salinibacter ruber DSM 13855	383	384
Methylobacterium chloromethanicum	385	386

Table 4b. Additional representative bacterial [2Fe-2S] DHAD proteins and encoding sequences.

Organism of derivation	Nucleic acid SEQ ID NO:	Amino acid SEQ ID NO:
Burkholderia ambifaria AMMD	387	388
Bradyrhizobium sp. BTAi1	389	390
Delftia acidovorans SPH-1	391	392
Microcystis aeruginosa NIES-843	393	394
uncultured marine microorganism HF4000_APKG8C21	395	396
Burkholderia ubonensis Bu	397	398
Gemmata obscuriglobus UQM 2246	399	400
Mycobacterium abscessus	401	402
Synechococcus sp. PCC 7002	403	404
Burkholderia graminis C4D1M	405	406
Methylobacterium radiotolerans JCM 2831	407	408
Leptothrix cholodnii SP-6	409	410
Verrucomicrobium spinosum DSM 4136	411	412
Cyanothece sp. ATCC 51142	413	414
Opitutus terrae PB90-1	415	416
Leptospira biflexa serovar Patoc strain 'Patoc 1 (Paris)'	417	418
Methylacidiphilum infernorum V4	419	420
Cupriavidus taiwanensis	421	422
Chthoniobacter flavus Ellin428	423	424
Cyanothece sp. PCC 7822	425	426
Phenylobacterium zucineum HLK1	427	428
Leptospirillum sp. Group II '5-way CG'	429	430
Arthrospira maxima CS-328	431	432
Oligotropha carboxidovorans OM5	433	434
Rhodospirillum centenum SW	435	436
Cyanothece sp. PCC 8801	437	438

Thermus aquaticus Y51MC23	439	440
Cyanothece sp. PCC 7424	441	442
Acidithiobacillus ferrooxidans ATCC 23270	443	444
Cyanothece sp. PCC 7425	445	446
Arthrobacter chlorophenolicus A6	447	448
Burkholderia multivorans CGD2M	449	450
Thermomicrobium roseum DSM 5159	451	452
bacterium Ellin514	453	454
Desulfobacterium autotrophicum HRM2	455	456
Thioalkalivibrio sp. K90mix	457	458
Flavobacteria bacterium MS024-3C	459	460
Flavobacteria bacterium MS024-2A	461	462
'Nostoc azollae' 0708	463	464
Acidobacterium capsulatum ATCC 51196	465	466
Gemmatimonas aurantiaca T-27	467	468
Gemmatimonas aurantiaca T-27	469	470
Rhodococcus erythropolis PR4	471	472
Deinococcus deserti VCD115	473	474
Rhodococcus opacus B4	475	476
Chryseobacterium gleum ATCC 35910	477	478
Thermobaculum terrenum ATCC BAA-798	479	480
Kribbella flavida DSM 17836	481	482
Gordonia bronchialis DSM 43247	483	484
Geodermatophilus obscurus DSM 43160	485	486
Xylanimonas cellulosilytica DSM 15894	487	488
Sphingobacterium spiritivorum ATCC 33300	489	490
Meiothermus silvanus DSM 9946	491	492
Meiothermus ruber DSM 1279	493	494
Nakamurella multipartita DSM 44233	495	496
Cellulomonas flavigena DSM 20109	497	498
Rhodothermus marinus DSM 4252	499	500
Planctomyces limnophilus DSM 3776	501	502
Beutenbergia cavernae DSM 12333	503	504
Spirosoma linguale DSM 74	505	506
Sphaerobacter thermophilus DSM 20745	507	508
Lactococcus lactis	509	510
Thermus thermophilus HB8	511	512
Anabaena variabilis ATCC 29413	513	514

Roseovarius sp. 217	515	516
uncultured *Prochlorococcus marinus* clone HF10-88D1	517	518
Burkholderia xenovorans LB400	519	520
Saccharomonospora viridis DSM 43017	521	522
Pedobacter heparinus DSM 2366	523	524
Microcoleus chthonoplastes PCC 7420	525	526
Acidimicrobium ferrooxidans DSM 10331	527	528
Rhodobacterales bacterium HTCC2083	529	530
Candidatus Pelagibacter sp. HTCC7211	531	532
Chitinophaga pinensis DSM 2588	533	534
Alcanivorax sp. DG881	535	536
Micrococcus luteus NCTC 2665	537	538
Verrucomicrobiae bacterium DG1235	539	540
Synechococcus sp. PCC 7335	541	542
Brevundimonas sp. BAL3	543	544
Dyadobacter fermentans DSM 18053	545	546
gamma proteobacterium NOR5-3	547	548
gamma proteobacterium NOR51-B	549	550
Cyanobium sp. PCC 7001	551	552
Jonesia denitrificans DSM 20603	553	554
Brachybacterium faecium DSM 4810	555	556
Paenibacillus sp. JDR-2	557	558
Octadecabacter antarcticus 307	559	560
Variovorax paradoxus S110	561	562

Table 5. SEQ ID NOs of Representative Fungal and Plant [2Fe-2S] DHAD Proteins and Encoding Sequences.

Description	SEQ ID NO: Nucleic acid	SEQ ID NO: Peptide
Schizosaccharomyces pombe ILV3	563	564
Saccharomyces cerevisiae ILV3	565	566
Kluyveromyces lactis ILV3	567	568
Candida albicans SC5314 ILV3	569	570
Pichia stipitis CBS 6054 ILV3	571	572
Yarrowia lipolytica ILV3	573	574
Candida galbrata CBS 138 ILV3	575	576
Chlamydomonas reinhardtii	577	578

Ostreococcus lucimarinus CCE9901	579	580
Vitis vinifera (Unnamed protein product: CAO71581.1)	581	582
Vitis vinifera (Hypothetical protein: CAN67446.1)	583	584
Arabidopsis thaliana	585	586
*Oryza sativa (*indica cultivar-group)	587	588
Physcomitrella patens subsp. Patens	589	590
Chaetomium globosum CBS 148.51	591	592
Neurospora crassa OR74A	593	594
Magnaporthe grisea 70-15	595	596
Gibberella zeae PH-1	597	598
Aspergillus niger	599	600
Neosartorya fischeri NRRL 181 (XP_001266525.1)	601	602
Neosartorya fischeri NRRL 181 (XP_001262996.1)	603	604
Aspergillus niger (hypothetical protein An03g04520)	605	606
Aspergillus niger (Hypothetical protein An14g03280)	607	608
Aspergillus terreus NIH2624	609	610
Aspergillus clavatus NRRL 1	611	612
Aspergillus nidulans FGSC A4	613	614
Aspergillus oryzae	615	616
Ajellomyces capsulatus NAm1	617	618
Coccidioides immitis RS	619	620
Botryotinia fuckeliana B05.10	621	622
Phaeosphaeria nodorum SN15	623	624
Pichia guilliermondii ATCC 6260	625	626
Debaryomyces hansenii CBS767	627	628
Lodderomyces elongisporus NRRL YB-4239	629	630
Vanderwaltozyma polyspora DSM 70294	631	632
Ashbya gossypii ATCC 10895	633	634
Laccaria bicolor S238N-H82	635	636
Coprinopsis cinerea okayama7#130	637	638
Cryptococcus neoformans var. neoformans JEC21	639	640
Ustilago maydis 521	641	642

Malassezia globosa CBS 7966	643	644
Aspergillus clavatus NRRL 1	645	646
Neosartorya fischeri NRRL 181 (Putative)	647	648
Aspergillus oryzae	649	650
Aspergillus niger (hypothetical protein An18g04160)	651	652
Aspergillus terreus NIH2624	653	654
Coccidioides immitis RS (hypothetical protein CIMG_04591)	655	656
Paracoccidioides brasiliensis	657	658
Phaeosphaeria nodorum SN15	659	660
Gibberella zeae PH-1	661	662
Neurospora crassa OR74A	663	664
Coprinopsis cinerea okayama 7#130	665	666
Laccaria bicolor S238N-H82	667	668
Ustilago maydis 521	669	670

Table 6. SEQ ID NOs of Representative [4Fe-4S] DHAD Proteins and Encoding Sequences.

Organism	SEQ ID NO: Nucleic acid	SEQ ID NO: Peptide
Escherichia coli str. K-12 substr. MG1655	671	672
Bacillus subtilis subsp. subtilis str. 168	673	674
Agrobacterium tumefaciens str. C58	675	676
Burkholderia cenocepacia MC0-3	677	678
Psychrobacter cryohalolentis K5	679	680
Psychromonas sp. CNPT3	681	682
Deinococcus radiodurans R1	683	684
Wolinella succinogenes DSM 1740	685	686
Zymomonas mobilis subsp. mobilis ZM4	687	688
Clostridium acetobutylicum ATCC 824	689	690
Clostridium beijerinckii NCIMB 8052	691	692
Pseudomonas fluorescens Pf-5	693	694
Methanococcus maripaludis C7	695	696
Methanococcus aeolicus Nankai-3	697	698
Vibrio fischeri ATCC 700601 (ES114)	699	700
Shewanella oneidensis MR-1 ATCC 700550	701	702

[0098] Additional [2Fe-2S] DHADs may be identified using the analysis described in U.S. Patent Appl. No. 12/569,636, filed Sept. 29, 2009, which is herein incorporated by reference. The analysis is as follows: A Profile Hidden Markov Model (HMM) was prepared based on amino acid sequences of eight functionally verified DHADs. The application of Profile HMM has been described. *See, e.g.*, Krogh *et al.*, *J. Mol. Biol. 235*:1501-1531 (1994) and Durbin *et al.*, "Markov chains and hidden Markov models," in Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids, Cambridge University Press (1998). A Profile HMM is a statistical model built of multiple sequence alignments that can be used to determine whether or not a test sequence belongs to a particular family of sequences. *See id.* A Profile HMM can be built by first generating an alignment of functionally verified sequences using conventional sequence alignment tools. Next, the sequence alignment is used to build the Profile HMM using publicly available software programs (*e.g.*, HMMER) that use a position-specific scoring system to capture information about the degree of conservation at various amino acid positions in the multiple alignment of the input sequences. More specifically, the scores of amino acid residues in a "match" state (*i.e.*, match state emission scores), or in an "insert" state (*i.e.*, insert state emission scores) are captured which are proportional to the expression: Log__2 (p_x)/(null_x). *See id.* In this expression, the term "p_x" is the probability of an amino acid residue, at a particular position in the alignment, according to the Profile HMM, and the term "null_x" is the probability according to the Null model. *See id.* The Null model is a simple one state probabilistic model with a pre-calculated set of emission probabilities for each of the amino acids derived from the distribution of amino acids. *See id.* "State" transition scores are also calculated as log odds parameters and are proportional to Log__2 (t_x). *See id.* In this expression, the term "t_x" is the probability of transiting to an emitter or non-emitter state. *See id.* Further details regarding the particular statistical analyses to generate a Profile HMM are available in Krogh *et al.*, *J. Mol. Biol. 235*:1501-1531 (1994) and Durbin *et al.*, "Markov chains and hidden Markov models," in Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids, Cambridge University Press (1998), and U.S. Patent Appl. No. 12/569,636.

[0099] A Profile Hidden Markov Model (HMM) was prepared based on amino acid sequences of eight functionally verified DHADs are from *Nitrosomonas europaea* (DNA

SEQ ID NO:309; protein SEQ ID NO:310), *Synechocystis sp.* PCC6803 (DNA SEQ ID:297; protein SEQ ID NO:298), *Streptococcus mutans* (DNA SEQ ID NO:167; protein SEQ ID NO:168), *Streptococcus thermophilus* (DNA SEQ ID NO:163; SEQ ID No:164), *Ralstonia metallidurans* (DNA SEQ ID NO:345; protein SEQ ID NO:346), *Ralstonia eutropha* (DNA SEQ ID NO:343; protein SEQ ID NO:344), and *Lactococcus lactis* (DNA SEQ ID NO:231; protein SEQ ID NO:232). In addition the DHAD from *Flavobacterium johnsoniae* (DNA SEQ ID NO:229; protein SEQ ID NO:230) was found to have dihydroxy-acid dehydratase activity when expressed in *E. coli* and was used in making the Profile. The Profile HMM is prepared 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). The output of the HMMER software program is a Profile Hidden Markov Model (HMM) that characterizes the input sequences. The Profile HMM prepared for the eight DHAD proteins is given in U.S. Appl. No. 12/569,636, filed Sept. 29, 2009 and in Table 12.

[00100] The first line in Table 12 for each position reports the probability for each amino acid to be in that "state" (match state emission scores). The second line reports the insert state emission scores, and the third line reports the state transition scores. The highest probability is highlighted for each position. These scores can be converted into "E values" (expectation values), which are the number of hits or matches to the Profile HMM one would expect to obtain just by chance. A protein having an E value of $< 10^{-5}$ match to the Profile HMM, indicates that the protein shares significant sequence similarity with the seed proteins used to construct the Profile HMM and that the protein belongs to the family represented by the profile HMM.

[0100] Any protein that matches the Profile HMM with an E value of $< 10^{-5}$ is a DHAD related protein, which includes [4Fe-4S] DHADs, [2Fe-2S] DHADs, arabonate dehydratases, and phosphogluconate dehydratases. In embodiments, sequences matching the Profile HMM are then analyzed for the presence of the three conserved cysteines, corresponding to positions 56, 129, and 201 in the *Streptococcus mutans* DHAD. The presence of all three conserved cysteines is characteristic of proteins having a [2Fe-2S]

cluster. Proteins having the three conserved cysteines include arabonate dehydratases and [2Fe-2S] DHADs. The [2Fe-2S] DHADs may be distinguished from the arabonate dehydratases by analyzing for signature conserved amino acids found to be present in the [2Fe-2S] DHADs or in the arabonate dehydratases at positions corresponding to the following positions in the *Streptococcus mutans* DHAD amino acid sequence. These signature amino acids are in [2Fe-2S] DHADs or in arabonate dehydratases, respectively, at the following positions (with greater than 90% occurance): 88 asparagine vs. glutamic acid; 113 not conserved vs. glutamic acid; 142 arginine or asparagine vs. not conserved; 165 not conserved vs. glycine; 208 asparagine vs. not conserved; 454 leucine vs. not conserved; 477 phenylalanine or tyrosine vs. not conserved; and 487 glycine vs. not conserved.

[0101] Additionally, the sequences of DHAD coding regions provided herein may be used to identify other homologs in nature. Such methods are well-known in the art, and various methods that may be used to isolate genes encoding homologous proteins are described in U.S. Appl. No. 12/569,636, filed Sept. 29, 2009, which such methods are incorporated by reference herein.

[0102] The presence of DHAD activity in a cell engineered to express a heterologous DHAD can be confirmed using methods known in the art. As one example, and as demonstrated in the Examples herein, crude extracts from cells engineered to express a bacterial DHAD may be used in a DHAD assay as described by Flint and Emptage (*J. Biol. Chem.* (1988) 263(8): 3558-64) using dinitrophenylhydrazine. In another example, DHAD activity may be assayed by expressing a heterologous DHAD identifiable by the methods disclosed herein in a yeast strain that lacks endogenous DHAD activity. If DHAD activity is present, the yeast strain will grow in the absence of branched-chain amino acids. DHAD activity may also be confirmed by more indirect methods, such as by assaying for a downstream product in a pathway requiring DHAD activity. Any product that has α-ketoisovalerate or α-ketomethylvalerate as a pathway intermediate may be measured in an assay for DHAD activity. A list of such products includes, but is not limited to, valine, isoleucine, leucine, pantothenic acid, 2-methyl-1-butanol, 3-methyl-1-butanol, and isobutanol.

[0103] Over-Expression of DHAD Activity

[0104] Applicants have found that expression of a heterologous DHAD can provide DHAD activity when expressed in a host cell. Expression of a DHAD which may be identified as described herein can provide DHAD activity for a biosynthetic pathway that includes conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate or 2,3-dihydroxymethylvalerate to α-ketomethylvalerate. In addition, the *S. mutans* [2Fe-2S] DHAD was shown in related U.S. Appl. No. 12/569,636, filed Sept. 29, 2009, incorporated by reference herein, to have higher stability in air as compared to the sensitivity in air of the E. coli [4Fe-4S] DHAD, which is desirable for obtaining better activity in a heterologous host cell.

[0105] Furthermore, as described herein, it has been found that expressing a heterologous DHAD protein at higher levels can provide increased DHAD activity when expressed in a host cell. High expression of a recombinant polynucleotide can be accomplished in at least two ways: 1) by increasing the copy number of a plasmid comprising the recombinant polynucleotide; or 2) by integrating multiple copies of the gene of interest into the host cell's chromosome. As exemplified herein, expression of multiple copies of the heterologous DHAD, provides an increase in specific activity of heterologous DHAD

[0106] Recombinant polynucleotides are typically cloned for expression using the coding sequence as part of a chimeric gene used for transformation, which includes a promoter operably linked to the coding sequence as well as a ribosome binding site and a termination control region. The coding region may be from the host cell for transformation and combined with regulatory sequences that are not native to the natural gene encoding DHAD. Alternatively, the coding region may be from another host cell.

[0107] Vectors useful for the transformation of a variety of host cells are common and described in the literature. Typically the vector contains a selectable marker and sequences allowing autonomous replication or chromosomal integration in the desired host. In addition, suitable vectors may 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 genes that are not native to the specific species chosen as a production host.

[0108] Yeast cells that can be hosts for expression or over-expression of a heterologous bacterial DHAD are any yeast cells that are amenable to genetic manipulation and include, but are not limited to, *Saccharomyces, Schizosaccharomyces, Hansenula, Candida, Kluyveromyces, Yarrowia, Issatchenkia*, and *Pichia*. Suitable strains include, but are not limited to, *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces thermotolerans, Candida glabrata, Candida albicans, Pichia stipitis* and *Yarrowia lipolytica*. In one embodiment, the host is *Saccharomyces cerevisiae*.

[0109] Expression is achieved by transforming a host cell with a gene comprising a sequence encoding DHAD, for example, a DHAD listed in Tables 4a, 4b, 5 or 6, or identified using the screening methods in related U.S. Appl. No. 12/569,636, filed Sept. 29, 2009, incorporated by reference herein. The coding region for the DHAD to be expressed may be codon optimized for the target host cell, as well known to one skilled in the art. Methods for gene expression in yeast are known in the art (see, e.g., Methods in Enzymology, Volume 194, Guide to Yeast Genetics and Molecular and Cell Biology (Part A, 2004, Christine Guthrie and Gerald R. Fink (Eds.), Elsevier Academic Press, San Diego, CA). Expression of genes in yeast typically requires a promoter, operably linked to a coding region of interest, and a transcriptional terminator. A number of yeast promoters can be used in constructing expression cassettes for genes in yeast, 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, GPM, and AOX1. Suitable transcriptional terminators include, but are not limited to, FBAt, GPDt, GPMt, ERG10t, GAL1t, CYC1, and ADH1.

[0110] Suitable promoters, transcriptional terminators, and DHAD coding regions may be cloned into *E. coli*-yeast shuttle vectors, and transformed into yeast cells. These vectors allow strain propagation in both *E. coli* and yeast strains. In one embodiment, the vector used contains a selectable marker and sequences allowing autonomous replication or chromosomal integration in the desired host. Examples of plasmids used in yeast are shuttle vectors pRS423, pRS424, pRS425, and pRS426 (American Type Culture Collection, Manassas, VA), which contain an *E. coli* replication origin (*e.g.*, pMB1), a yeast 2-micron origin of replication, and a marker for nutritional selection. The selection markers for these four vectors are His3 (vector pRS423), Trp1 (vector pRS424), Leu2

(vector pRS425) and Ura3 (vector pRS426). Construction of expression vectors with a chimeric gene encoding the described DHADs can be performed by either standard molecular cloning techniques in *E. coli* or by the gap repair recombination method in yeast.

[0111] The gap repair cloning approach takes advantage of the highly efficient homologous recombination in yeast. For example, a yeast vector DNA is digested (*e.g.*, in its multiple cloning site) to create a "gap" in its sequence. A number of insert DNAs of interest are generated that contain a $\geq$ 21 bp sequence at both the 5' and the 3' ends that sequentially overlap with each other, and with the 5' and 3' terminus of the vector DNA. For example, to construct a yeast expression vector for "Gene X," a yeast promoter and a yeast terminator are selected for the expression cassette. The promoter and terminator are amplified from the yeast genomic DNA, and Gene X is either PCR amplified from its source organism or obtained from a cloning vector comprising Gene X sequence. There is at least a 21 bp overlapping sequence between the 5' end of the linearized vector and the promoter sequence, between the promoter and Gene X, between Gene X and the terminator sequence, and between the terminator and the 3' end of the linearized vector. The "gapped" vector and the insert DNAs are then co-transformed into a yeast strain and plated on the medium containing the appropriate compound mixtures that allow complementation of the nutritional selection markers on the plasmids. The presence of correct insert combinations can be confirmed by PCR mapping using plasmid DNA prepared from the selected cells. The plasmid DNA isolated from yeast (usually low in concentration) can then be transformed into an *E. coli* strain, *e.g. TOP10*, followed by mini preps and restriction mapping to further verify the plasmid construct. Finally, the construct can be verified by sequence analysis.

[0112] Like the gap repair technique, integration into the yeast genome also takes advantage of the homologous recombination system in yeast. For example, a cassette containing a coding region plus control elements (promoter and terminator) and auxotrophic marker is PCR-amplified with a high-fidelity DNA polymerase using primers that hybridize to the cassette and contain 40-70 base pairs of sequence homology to the regions 5' and 3' of the genomic area where insertion is desired. The PCR product is then transformed into yeast and plated on medium containing the appropriate compound mixtures that allow selection for the integrated auxotrophic marker. For example, to

integrate "Gene X" into chromosomal location "Y", the promoter-coding regionX-terminator construct is PCR amplified from a plasmid DNA construct and joined to an autotrophic marker (such as *URA3*) by either SOE PCR or by common restriction digests and cloning. The full cassette, containing the promoter-coding regionX-terminator-*URA3* region, is PCR amplified with primer sequences that contain 40-70 bp of homology to the regions 5' and 3' of location "Y" on the yeast chromosome. The PCR product is transformed into yeast and selected on growth media lacking uracil. Transformants can be verified either by colony PCR or by direct sequencing of chromosomal DNA.

[0113] In addition to the above materials and methods that may be used to express a heterologous DHAD, these same, or similar, materials and methods may be used to over-express a heterologous DHAD using modifications known to one of skill in the art. For example, when using a plasmid-based system to over-express the recombinant polynucleotide, a high-copy number vector, or a vector with a copy number that can be regulated, may be constructed. Such a regulatable or inducible system is described herein in Example 1; however, other systems are known to one of skill in the art and may be used to construct other high-copy number or copy number regulatable vectors. Alternatively, when using an integration-based system to over-express the recombinant polypeptide, an integration vector is required for targeting at multiple integration sites. A multiple integration-based system is described herein in Example 2; however, other multiple integration-based systems are known to one of skill in the art and may be used to target multiple integrations of a recombinant polypeptide, for example integration into rDNA regions.

[0114] Expression of the heterologous DHAD in the recombinant host cell can be quantified, *e.g.*, by a percent of total cell protein. Such over-expression can be quantified in an amount selected from the group consisting of: (a) greater than about 0.001% of total cell protein; (b) greater than about 0.01% of total cell protein; (c) greater than about 0.1% of total cell protein; (d) greater than about 0.5% of total cell protein; (e) greater than about 1.0% of total cell protein; (f) greater than about 2.0% of total cell protein; (g) greater than about 5% of total cell protein; (h) greater than about 10% of total cell protein; and (i) greater than about 20% of total cell protein.

[0115] The specific activity of the heterologous DHAD produced in a recombinant host cell can be quantified, *e.g.*, as U/mg. The heterologous DHAD specific activity can be

selected from the group consisting of: (a) greater than about 0.25 U/mg; (b) greater than about 0.3 U/mg; (c) greater than about 0.5 U/mg; (d) greater than about 1.0 U/mg; (e) greater than about 1.5 U/mg; (f) greater than about 2.0 U/mg; (g) greater than about 3.0 U/mg; (h) greater than about 4.0 U/mg; (i) greater than about 5.0 U/mg; (j) greater than about 6.0 U/mg; (k) greater than about 7.0 U/mg; (l) greater than about 8.0 U/mg; (m) greater than about 9.0 U/mg; (n) greater than about 10.0 U/mg; (o) greater than about 20.0 U/mg; and (p) greater than about 50.0 U/mg.

[0116] The heterologous DHAD specific activity can also be quantified, e.g., as a percent comparison to an endogenous DHAD specific activity or to some other control DHAD specific activity. An example of a "control" DHAD specific activity is that from a heterologous DHAD expressed in a recombinant host cell using a low copy number plasmid or a plasmid that is not other wise inducible or regulatable. Such a control establishes a baseline from which to compare the specific activity of the same heterologous DHAD expressed in a recombinant host cell using a high copy number plasmid or a plasmid with copy number that can be regulated, or co-expressed with polynucleotides encoding polypeptides affecting Fe-S cluster biosynthesis or Fe uptake and utilization, as described below. Thus, the increase in specific activity of the heterologous DHAD when compared to the control DHAD specific activity can be in an amount selected from the group consisting of: greater than an about 10% increase; greater than an about 20% increase; greater than an about 30% increase; greater than an about 40% increase; greater than an about 50% increase; greater than an about 60% increase; greater than an about 70% increase; greater than an about 80% increase; greater than an about 90% increase; greater than an about 95% increase; greater than an about 98% increase; and greater than an about 99% increase. The heterologous DHAD specific activity can also be expressed by “fold increase” over control. Thus, the increase in specific activity can be selected from the group consisting of: (a) greater than about 2-fold higher, (b) greater than about 5-fold higher, (c) greater than about 8-fold higher, or (d) greater than about 10-fold higher than control.

[0117] Fe-S Cluster Forming Proteins and Fe Regulation, Utilization, and Homeostasis

[0118] As described above, DHAD enzymes require Fe-S clusters for functioning, therefore, they must be expressed in a host having the genetic machinery to produce and load Fe-S clusters into the apo-protein if they are going to be expressed in functional

form. As described elsewhere herein, in normal yeast, the mitochondria play an important role in Fe-S cluster biosynthesis. The flux in the formation and movement of Fe-S cluster precursors from mitochondria to Fe-S cluster requiring proteins in the cytosol of normal yeast is believed to be limited. For example, after a point a further increase in the expression of the protein of heterologous DHADs in the cytosol does not result in a corresponding increase in DHAD activity. While not wishing to be bound by theory, it is believed that this is because the increased amounts of the heterologous DHAD are not getting loaded with the Fe-S cluster requisite for activity because the cell is not able to supply the increased demand for Fe-S clusters that arises in the conditions described above. Demonstrated herein is that yeast cells can be genetically modified in 2 ways (separately or contemporaneously) that will result in an increased fraction of the heterologous DHAD expressed in the cytosol being loaded with its requisite Fe-S cluster. One way is to to modify the expression of yeast genes involved in the Fe-S cluster formation, such as Fe-S cluster biosynthesis pathway genes or Fe uptake and utilization genes. The other way is to express heterologous genes involved in Fe-S cluster biosynthesis or Fe uptake and utilization in the cytoplasm of yeast.

[0119] Yeast genes that encode polypeptides that are involved in Fe uptake and utilization and Fe-S cluster biosynthesis are candidates for modification of expression. In embodiments, the modification results in increased function of a selected Fe-S cluster requiring protein.

[0120] As an example, Aft1 has been found to act as a transcriptional activator for genes into the iron regulon (Kumanovics, *et al. J. Biol. Chem.*, 2008. 283, p. 10276-10286; Li, H., et al., *The Yeast Iron Regulatory Proteins Grx3/4 and Fra2 form Heterodimeric Complexes Containing a [2Fe-2S] Cluster with Cysteinyl and Histidyl Ligation.* Biochemistry, 2009. **48**(40): p. 9569-9581. As exemplified herein, the deletion of known inhibitors of Aft1 translocation, results in an increase in specific activity of an Fe-S cluster requiring protein because it leads to an increase Fe-S cluster loading of the protien. While not wishing to be bound by theory, it is thus believed that altering expression of certain genes of the Fe regulon, whether directly or through deletion or upregulation of inhibitors, will likewise increase the loading and function of Fe-S cluster requiring proteins. For example, genes that play a role in, or are part of, Fe utilization and homeostasis in yeast, such as Fe Regulon genes, may be targeted for altered

expression. Such genes are known in the art, and examples of these genes are listed in Table 7. (The list in Table 7 is taken from Rutherford, J.C., *et al.*, *Activation of the Iron Regulon by the Yeast Aft1/Aft2 Transcription Factors Depends on Mitochondrial but Not Cytosolic Iron-Sulfur Protein Biogenesis., J. Biol. Chem.*, 2005. **280**(11): p. 10135-10140; Foury, F. and D. Talibi, *Mitochondrial control of iron homeostasis. A genome wide analysis of gene expression in a yeast frataxin-deficient strain. J. Biol. Chem.*, 2001. **276**(11): p. 7762-7768; and Shakoury-Elizeh, M., *et al.*, *Transcriptional remodeling in response to iron deprivation in Saccharomyces cerevisiae. Mol. Biol. Cell*, 2004. **15**(3): p. 1233-1243.)

Table 7. Examples of yeast genes associated with Fe uptake and utilization .

Gene Name	Putative Function	Nucleic Acid	Amino Acid
		SEQ ID NO:	SEQ ID NO:
ARN1	Transporter, member of the ARN family of transporters that specifically recognize siderophore-iron chelates; responsible for uptake of iron bound to ferrirubin, ferrirhodin, and related siderophores	805	738
ARN2	Transporter, member of the ARN family of transporters that specifically recognize siderophore-iron chelates; responsible for uptake of iron bound to the siderophore triacetylfusarinine C	806	739
ATX1	Cytosolic copper metallochaperone that transports copper to the secretory vesicle copper transporter Ccc2p for eventual insertion into Fet3p, which is a multicopper oxidase required for high-affinity iron uptake	802	735
CCC2	Cu(+2)-transporting P-type ATPase, required for export of copper from the cytosol into an extracytosolic compartment; has similarity to human proteins involved in Menkes and Wilsons diseases	803	736
COT1	Vacuolar transporter that mediates zinc transport into the vacuole; overexpression confers resistance to cobalt and rhodium	816	749
ENB1 (ARN4)	Endosomal ferric enterobactin transporter, expressed under conditions of iron deprivation; member of the major facilitator superfamily; expression is regulated by Rcs1p and affected by chloroquine treatment	808	741
FET3	Ferro-O2-oxidoreductase required for high-affinity iron uptake and involved in mediating resistance to copper ion toxicity, belongs to class of integral membrane multicopper oxidases	800	733
FET5	Multicopper oxidase, integral membrane protein with similarity to Fet3p; may have a role in iron transport	814	747
FIT1	Mannoprotein that is incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor, involved in the retention of siderophore-iron in the cell wall	792	725
FIT2	Mannoprotein that is incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor, involved in the	793	726

	retention of siderophore-iron in the cell wall		
FIT3	Mannoprotein that is incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor, involved in the retention of siderophore-iron in the cell wall	794	727
FRE1	Ferric reductase and cupric reductase, reduces siderophore-bound iron and oxidized copper prior to uptake by transporters; expression induced by low copper and iron levels	795	728
FRE2	Ferric reductase and cupric reductase, reduces siderophore-bound iron and oxidized copper prior to uptake by transporters; expression induced by low copper and iron levels	796	729
FRE3	Ferric reductase, reduces siderophore-bound iron prior to uptake by transporters; expression induced by low iron levels	797	730
FRE4	Ferric reductase, reduces a specific subset of siderophore-bound iron prior to uptake by transporters; expression induced by low iron levels	798	731
FRE5	Putative ferric reductase with similarity to Fre2p; expression induced by low iron levels; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies	799	732
FRE6	Putative ferric reductase with similarity to Fre2p; expression induced by low iron levels	817	750
FTH1	Putative high affinity iron transporter involved in transport of intravacuolar stores of iron; forms complex with Fet5p; expression is regulated by iron; proposed to play indirect role in endocytosis	813	746
FTR1	High affinity iron permease involved in the transport of iron across the plasma membrane; forms complex with Fet3p; expression is regulated by iron	801	734
HMX1	ER localized, heme-binding peroxidase involved in the degradation of heme; does not exhibit heme oxygenase activity despite similarity to heme oxygenases; expression regulated by AFT1	823	756
SIT1 (*ARN3*)	Ferrioxamine B transporter, member of the ARN family of transporters that specifically recognize siderophore-iron chelates; transcription is induced during iron deprivation and diauxic shift; potentially phosphorylated by Cdc28p	807	740
SMF3	Putative divalent metal ion transporter involved in iron homeostasis; transcriptionally regulated by metal ions; member of the Nramp family of metal transport proteins	815	741
TIS11 (*CTH2*)	mRNA-binding protein expressed during iron starvation; binds to a sequence element in the 3'-untranslated regions of specific mRNAs to mediate their degradation; involved in iron homeostasis	824	757
VHT1	High-affinity plasma membrane H+-biotin (vitamin H) symporter; mutation results in fatty acid auxotrophy; 12 transmembrane domain containing major facilitator subfamily member; mRNA levels negatively regulated by iron deprivation and biotin	822	755

[0121] Based on their functions and association with Fe uptake and utilization, the proteins encoded by the genes disclosed in Table 7 are candidates for affecting Fe-S cluster biosynthesis. Additional yeast genes associated with Fe uptake and utilization or Fe-S cluster biosynthesis include those listed in Table 8.

Table 8. Genes Associated With Yeast Fe Uptake and Utilization or Fe-S Cluster Biosynthesis

Gene Name	Nucleic Acid SEQ ID NO:	Amino Acid SEQ ID NO:	Putative Function
AFT1	770	703	Transcription factor involved in iron utilization and homeostasis; binds the consensus site PyPuCACCCPu and activates the expression of target genes in response to changes in iron availability
AFT2	771	704	Iron-regulated transcriptional activator; activates genes involved in intracellular iron use and required for iron homeostasis and resistance to oxidative stress; similar to Aft1p
AIM1	779	712	Interacts with Grx3/4
ARH1	855	837	Oxidoreductase of the mitochondrial inner membrane, involved in cytoplasmic and mitochondrial iron homeostasis and required for activity of Fe-S cluster-containing enzymes; one of the few mitochondrial proteins essential for viability (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
ATM1	830	763	Mitochondrial inner membrane ATP-binding cassette (ABC) transporter, exports mitochondrially synthesized precursors of iron-sulfur (Fe/S) clusters to the cytosol (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
BUD32	778	711	Interacts with Grx3/4 and Aft1p
CAD1 (YAP2)	791	724	Stress responses including Fe deprivation; also regulates CTI6 and MRS4 genes
CCC1	811	744	Putative vacuolar Fe2+/Mn2+ transporter; suppresses respiratory deficit of yfh1 mutants, which lack the ortholog of mammalian frataxin, by preventing mitochondrial iron accumulation
CFD1	834	767	Highly conserved, iron-sulfur cluster binding protein localized in the cytoplasm; forms a complex with Nbp35p that is involved in iron-sulfur protein assembly in the cytosol (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
CIA1	836	769	WD40 repeat protein involved in assembly of cytosolic and nuclear iron-sulfur proteins; similar to the human Ciao1 protein; YDR267C is an essential gene (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
CMK1	784	717	Interacts with Grx4p
CTH1	825	758	mRNA binding and degradation under Fe depletion conditions
CTI6	786	719	Growth in low iron conditions
CYC8 (SSN6)	787	720	General transcriptional co-repressor, acts together with Tup1p; also acts as part of a transcriptional co-activator complex that recruits the SWI/SNF and SAGA complexes to promoters; can form the prion [OCT+]
DAP1	820	753	
DRE2	781	714	Interacts with Grx3p
ERV1	856	838	Flavin-linked sulfhydryl oxidase of the mitochondrial intermembrane space (IMS), oxidizes Mia40p as part of a disulfide relay system that promotes IMS retention of imported proteins; ortholog of human hepatopoietin (ALR) (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))

			Central players of the export pathway are the ABC transporter Atm1p of the mitochondrial inner membrane, the sulfhydryl oxidase Erv1p of the intermembrane space, and the tripeptide glutathione (23, 27, 50) (*see* Gerber, J., *et al.*, *Mol. Cell. Biol. 24*(11):4848-57 (2004))
ESA1	782	715	Interacts with Grx4p/Aft1p
FET4	809	742	Low-affinity Fe(II) transporter of the plasma membrane
FRA1	772	705	Protein involved in negative regulation of transcription of iron regulon; forms an iron independent complex with Fra2p, Grx3p, and Grx4p; cytosolic; mutant fails to repress transcription of iron regulon and is defective in spore formation
FRA2	773	706	Protein involved in negative regulation of transcription of iron regulon; forms an iron independent complex with Fra2p, Grx3p, and Grx4p; null mutant fails to repress iron regulon and is sensitive to nickel
GEF1	804	737	Copper transporter/loading for Fet3p
GGC1 (*YHM1*)	857	839	Mitochondrial GTP/GDP transporter, essential for mitochondrial genome maintenance; has a role in mitochondrial iron transport; member of the mitochondrial carrier family
GRX1	858	840	Hydroperoxide and superoxide-radical responsive heat-stable glutathione-dependent disulfide oxidoreductase with active site cysteine pair; protects cells from oxidative damage
GRX2	832	765	Cytoplasmic glutaredoxin, thioltransferase, glutathione-dependent disulfide oxidoreductase involved in maintaining redox state of target proteins, also exhibits glutathione peroxidase activity, expression induced in response to stress
GRX3	774	707	Hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase; monothiol glutaredoxin subfamily member along with Grx4p and Grx5p; protects cells from oxidative damage
GRX4	775	708	Hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase; monothiol glutaredoxin subfamily member along with Grx3p and Grx5p; protects cells from oxidative damage.
GRX5	831	764	Hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase; mitochondrial matrix protein involved in the synthesis/assembly of iron-sulfur centers; monothiol glutaredoxin subfamily member along with Grx3p and Grx4p (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
HDA1	790	723	Interacts with Tup1p, Ssn6p for Aft1/2p regulation in the absence of heme
IBA57	859	841	Mitochondrial matrix protein involved in the incorporation of iron-sulfur clusters into mitochondrial aconitase-type proteins; activates the radical-SAM family members Bio2p and Lip5p; interacts with Ccr4p in the two-hybrid system (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
ISA1	860	842	Mitochondrial matrix protein involved in biogenesis of the iron-sulfur (Fe/S) cluster of Fe/S proteins, isa1 deletion causes loss of mitochondrial DNA and respiratory deficiency; depletion reduces growth on nonfermentable carbon sources (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))

ISA2	861	843	Protein required for maturation of mitochondrial and cytosolic Fe/S proteins, localizes to the mitochondrial intermembrane space, overexpression of ISA2 suppresses grx5 mutations (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
ISU1	828	761	Conserved protein of the mitochondrial matrix, performs a scaffolding function during assembly of iron-sulfur clusters, interacts physically and functionally with yeast frataxin (Yfh1p); isu1 isu2 double mutant is inviable (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
ISU2	829	762	Conserved protein of the mitochondrial matrix, required for synthesis of mitochondrial and cytosolic iron-sulfur proteins, performs a scaffolding function in mitochondria during Fe/S cluster assembly; isu1 isu2 double mutant is inviable (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
JAC1	862	844	Specialized J-protein that functions with Hsp70 in Fe-S cluster biogenesis in mitochondria, involved in iron utilization; contains a J domain typical to J-type chaperones; localizes to the mitochondrial matrix (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
MGE1	863	845	Mitochondrial matrix cochaperone, acts as a nucleotide release factor for Ssc1p in protein translocation and folding; also acts as cochaperone for Ssq1p in folding of Fe-S cluster proteins; homolog of E. coli GrpE (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
MRS3	819	752	Iron transporter that mediates Fe2+ transport across the inner mitochondrial membrane; mitochondrial carrier family member, similar to and functionally redundant with Mrs4p; active under low-iron conditions; may transport other cations (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
MRS4	818	751	Iron transporter that mediates Fe2+ transport across the inner mitochondrial membrane; mitochondrial carrier family member, similar to and functionally redundant with Mrs3p; active under low-iron conditions; may transport other cations (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
MSN5	776	709	Exporting Aft1p and other proteins from the nucleus
NAR1	833	766	Component of the cytosolic iron-sulfur (FeS) protein assembly machinery, required for maturation of cytosolic and nuclear FeS proteins and for normal resistance to oxidative stress; homologous to human Narf (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
NBP35	835	768	Essential iron-sulfur cluster binding protein localized in the cytoplasm; forms a complex with Cfd1p that is involved in iron-sulfur protein assembly in the cytosol; similar to P-loop NTPases (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
NFS1	864	846	Cysteine desulfurase involved in iron-sulfur cluster (Fe/S) biogenesis; required for the post-transcriptional thio-modification of mitochondrial and cytoplasmic tRNAs; essential protein located predominantly in mitochondria (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
NFU1	865	847	Protein involved in iron utilization in mitochondria; similar to NifU, which is a protein required for the maturation of the Fe/S clusters of nitrogenase in

			nitrogen-fixing bacteria (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
NHP6a and b	788,789	721, 722	Both are high-mobility group non-histone chromatin protein, functionally redundant with Nhp6Bp; homologous to mammalian high mobility group proteins 1 and 2; acts to recruit transcription factor Rcs1p to certain promoters
PSE1	777	710	Importing Aft1p and other proteins to the nucleus
SMF1	810	743	Low affinity Fe(II) transporter of the plasma membrane
SNF1	866	848	AMP-activated serine/threonine protein kinase found in a complex containing Snf4p and members of the Sip1p/Sip2p/Gal83p family; required for transcription of glucose-repressed genes, thermotolerance, sporulation, and peroxisome biogenesis
SNF2	867	849	Catalytic subunit of the SWI/SNF chromatin remodeling complex involved in transcriptional regulation; contains DNA-stimulated ATPase activity; functions interdependently in transcriptional activation with Snf5p and Snf6p
SNF3	868	850	Plasma membrane glucose sensor that regulates glucose transport; has 12 predicted transmembrane segments; long cytoplasmic C-terminal tail is required for low glucose induction of hexose transporter genes HXT2 and HXT4
SNF4	869	851	Activating gamma subunit of the AMP-activated Snf1p kinase complex (contains Snf1p and a Sip1p/Sip2p/Gal83p family member); activates glucose-repressed genes, represses glucose-induced genes; role in sporulation, and peroxisome biogenesis
SSQ1 .	827	760	Mitochondrial hsp70-type molecular chaperone, required for assembly of iron/sulfur clusters into proteins at a step after cluster synthesis, and for maturation of Yfh1p, which is a homolog of human frataxin implicated in Friedreich's ataxia (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008)) .
TIM12 (*MRS5*)	871	853	Essential protein of the inner mitochondrial membrane, peripherally localized; component of the TIM22 complex, which is a twin-pore translocase that mediates insertion of numerous multispanning inner membrane protein.
TUP1	785	718	General repressor of transcription
NP_011911.1	821	754	
VPS41 (*FET2*)	872	854	Vacuolar membrane protein that is a subunit of the homotypic vacuole fusion and vacuole protein sorting (HOPS) complex; essential for membrane docking and fusion at the Golgi-to-endosome and endosome-to-vacuole stages of protein transport
YAH1	870	852	Ferredoxin of the mitochondrial matrix required for formation of cellular iron-sulfur proteins; involved in heme A biosynthesis; homologous to human adrenodoxin (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))
YAP5	812	745	Regulation (CCC1)
YFH1 (Frataxin)	826	759	Mitochondrial matrix iron chaperone; oxidizes and stores iron; interacts with Isu1p to promote Fe-S cluster assembly; mutation results in multiple Fe/S-dependent enzyme deficiencies; human frataxin homolog is mutated in Friedrich's ataxia (*see, e.g.*, Lill, R. and U. Muehlenhoff, Ann. Rev. Biochem. 77:669-700 (2008))

YRA1	783	716	Interacts with Grx4p
ZPR1	780	713	Interacts with Aft1p

[0122] Additional genes encoding polypeptides affecting Fe-S cluster biosynthesis from other host cells have been identified and include, but are not limited to, those genes listed in Table 9.

Table 9. Genes Directly Involved in Fe-S Cluster Biosynthesis from Various Cells

Gene Name SEQ ID NOs(Amino Acid, Nucleic Acid)	**Function (Accession; CDS)**
***Azotobacter vinelandii nif* genes** (Figures 6A and 6B; *see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005))	
iscA^{nif} *(873, 894)*	[Fe-S] cluster scaffold protein (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002797399.1; nucleotides 153037 to 153360 of NC_012560.1)
nifU (875, 896)	NifU is a scaffold protein for assembly and transfer of iron-sulfur clusters (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)). (YP_002797400.1; nucleotides 153425 to 154363 of NC_012560.1)
nifS (874, 895)	Cysteine desulfurase involved in the mobilization of S for nitrogenase maturation (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)). (YP_002797401.1; nucleotides 154365 to 155573 of NC_012560.1)
cysE1 (876, 897)	Involved in cysteine biosynthesis (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002797403.1; nucleotides 156797 to 157594 of NC_012560.1)
cysE2 (929, 947)	Involved in cysteine biosynthesis (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002801153.1; reverse complement of nucleotides 4092159 to 4092938 of NC_012560.1)
iscS (930, 948)	Cysteine desulfurase involved in the mobilization of S (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002801151.1; reverse complement of nucleotides of 4090290 to 4091504 of NC_012560.1)
iscU (931, 949)	[Fe-S] cluster scaffold protein (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002801150.1; reverse complement of nucleotides 4089860 to 4090246 of NC_012560.1)
iscA (932, 950)	[Fe-S] cluster scaffold protein (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005))

	(YP_002801149.1; reverse complement of nucleotides 4089511 to 4089834 of NC_012560.1)
hscB *(933, 951)*	HscB heat shock cognate protein associated with Isc-directed [Fe-S] protein maturation (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002801148.1; reverse complement of nucleotides 4088980 to 4089501 of NC_012560.1)
hscA *(934, 952)*	HscA heat shock cognate protein associated with Isc-directed [Fe-S] protein maturation (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002801147.1; reverse complement of nucleotides 4087072 to 4088937 of NC_012560.1)
Fdx *(935, 953)*	Ferredoxin (YP_002801146.1; reverse complement of nucleotides 4086730 to 4087071 of NC_012560.1)
sufS *(936, 954)*	Cysteine desulfurase involved in the mobilization of S (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002801025.1; nucleotides 3961166 to 3962515 of NC_012560.1)
sufE *(937, 955)*	(YP_002801026.1; nucleotides 3962512 to 3962916 of NC_012560.1)
cysE3 *(938, 956)*	Involved in cysteine biosynthesis (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002799274.1; nucleotides 2093069 to 2094052 of NC_012560.1)
sufS2 *(939, 957)*	Cysteine desulfurase involved in the mobilization of S (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002799276.1; nucleotides 2095267 to 2097081 of NC_012560.1)
iscA2 also known as *eprA* *(877, 898)*	[Fe-S] cluster scaffold protein (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)) (YP_002801687.1; reverse complement of nucleotides 4681573 to 4681923 of NC_012560.1)
Nfu also known as *NfuA* *(878, 899)*	Human nfu appears to be a persulfide reductase according to the equation shown in Figure 6C. (*see* Liu, Y., W. Qi, and J.A. Cowan, *Biochem. 48*(5):973-80 (2009)) (YP_002800022.1; reverse complement of nucleotides 2961161 to 2961745 of NC_012560.1)
nfuA also known as *AnfU* *(879, 900)*	Spectroscopic and analytical studies indicate that one [4Fe-4S] cluster can be assembled *in vitro* within a dimeric form of NfuA. The resultant [4Fe-4S] cluster-loaded form of NfuA is competent for rapid in vitro activation of apo-aconitase. Based on these results a model is proposed where NfuA could represent a class of intermediate [Fe-S] cluster carriers involved in [Fe-S] protein maturation. (*see* Bandyopadhyay, S., *et al.*, *J Biol. Chem. 283*(20):14092-99 (2008)) (YP_002801977.1; nucleotides 4963727 to 4964017 of NC_012560.1)
nfuV also known as *VnfU*	Could have specialized functions related to the maturation, protection, or repair of specific [Fe-S] proteins (*see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005)).

(880, 901)	(YP_002797514.1; reverse complement of nucleotides 263828 to 264118 of NC_012560.1)
***Helicobacter pylori nif* genes** (Figure 7; *see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005))	
nifS *(881, 902)*	NifS is a cysteine desulfurase. (YP_003057033.1; nucleotides 218891 to 220054 of NC_012973.1)
nifU *(882, 903)*	NifU is a scaffold protein for assembly and transfer of iron-sulfur clusters. (YP_003057034.1; nucleotides 220076 to 221056 of NC_012973.1)
nfu *(927, 945)*	(YP_003058109.1; nucleotides 1448886 to 1449155 of NC_012973.1)
iscS *(928, 946)*	(YP_003057709.1; reverse complement of nucleotides 1012615 to 1013937 of NC_012973.1)
***E. coli isc* genes** (Figure 8; *see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005))	
iscS *(883, 904)*	**EcoCyc:** IscS is a cysteine desulfurase that catalyzes the conversion of cysteine into alanine and sulfur via intermediate formation of a cysteine persulfide. (YP_026169.1; reverse complement of nucleotides 2658339 to 2659553 of NC_000913.2)
iscU *(884, 905)*	**EcoCyc:** IscU is a scaffold protein for assembly and transfer of iron-sulfur clusters. IscU is able to form 2Fe-2S clusters and transfer them to apo-ferredoxin, acting catalytically. The chaperones HscA and HscB and ATP hydrolysis by HscA accelerate cluster transfer. (NP_417024.1; reverse complement of nucleotides 2657925 to 2658311 of NC_000913.2)
iscA *(885, 906)*	**EcoCyc:** IscA is an iron-sulfur cluster assembly protein that forms the [2Fe-2S] cluster of ferredoxin. It has been shown to bind iron with an apparent association constant of 3x10-19 M^{-1}. *In vitro* in the presence of IscS and cysteine, IscA can provide iron to iscU. Native [2Fe-2S] SufA can transfer its Fe-S cluster to both [2Fe-2S] and [4Fe-4S] apoproteins. (*see* Gupta, V., *et al.*, *J. Am. Chem. Soc. 131*(17):6149-53 (2009)) The results suggest that the biogenesis of the [4Fe-4S] clusters and the [2Fe-2S] clusters may have distinct pathways and that IscA/SufA paralogues are essential for the [4Fe-4S] cluster assembly, but are dispensable for the [2Fe-2S] cluster assembly in E. coli under aerobic conditions. (Tan, G., *et al.*, *Biochem. J.*, 420(3):463-72 (2009)) (NP_417023.1; reverse complement of nucleotides 2657585 to 2657908 of NC_000913.2)
hscB *(886, 907)*	**EcoCyc:** HscB is a co-chaperone that stimulates HscA (Hsc66) ATPase activity. HscB does not exhibit its own chaperone activity. HscB is required for wild-type stimulation of HscA ATPase activity by the

	substrate, IscU, and for wild-type interaction between HscA and IscU. This system is involved in iron-sulfur cluster assembly. (NP_417022.1; reverse complement of nucleotides 2656974 to 2657489 of NC_000913.2)
hscA *(887, 908)*	**EcoCyc:** Hsc66 together with Hsc20 may comprise a chaperone system similar to DnaK/DnaJ. Hsc66 is required for the assembly of iron-sulfur clusters. IscU may be a substrate for Hsc66. In the presence of Hsc20, IscU stimulates the ATPase activity of Hsc66 up to 480-fold; the *in vivo* turnover rate of the chaperone cycle may be determined by the availability of the IscU-Hsc20 complex. Hsc66 directly interacts with IscU, IscA, and Fdx. (NP_417021.1; reverse complement of nucleotides 2655107 to 2656957 of NC_000913.2)
Fdx *(888, 909)*	**EcoCyc:** [2Fe-2S] ferridoxin (NP_417020.1; reverse complement of nucleotides 2654770 to 2655105 of NC_000913.2)
***E. coli suf* genes** (Figure 9; *see* Johnson, D.C., *et al.*, *Ann. Rev. Biochem. 74*:247-81 (2005))	
sufA *(889, 910)*	**EcoCyc:** SufA is part of the protein machinery that is involved in the biosynthesis of iron-sulfur clusters. *In vitro*, purified apoSufA can chelate iron-sulfur clusters by treatment with iron and sulfide under anaerobic conditions. HoloSufA then can form a fast and tight association with the target apoprotein biotin synthase (BioB) and transfers a [4Fe-4S] cluster to BioB in a slow reaction. (NP_416199.1; reverse complement of nucleotides 1762042 to 1762410 of NC_000913.2)
sufB *(890, 911)*	**EcoCyc:** The SufB-SufC-SufD complex activates the cysteine desulfurase activity SufS in conjunction with the SufE sulfur acceptor protein. (NP_416198.2; reverse complement of nucleotides 1760546 to 1762033 of NC_000913.2)
sufC *(891, 912)*	**EcoCyc:** SufC is part of the protein machinery that is involved in the biosynthesis of iron-sulfur clusters. The SufB-SufC-SufD complex activates the cysteine desulfurase activity of SufS in conjunction with the SufE sulfur acceptor protein. (NP_416197.1; reverse complement of nucleotides 1759790 to 1760536 of NC_000913.2)
sufD *(892, 913)*	**EcoCyc:** The SufB-SufC-SufD complex activates the cysteine desulfurase activity SufS in conjunction with the SufE sulfur acceptor protein (NP_416196.1; reverse complement of nucleotides 1758544 to 1759815 of NC_000913.2)
sufS *(893, 914)*	**EcoCyc:** SufS is a member of the NifS protein family. SufS exhibits activity with respect to assembly of the ferredoxin iron-sulfur cluster in an *in vitro* assay. (NP_416195.1; reverse complement of nucleotides 1757327 to 1758547 of NC_000913.2)

sufE1 also known as suf E (925, 943)	(NP_416194.1; reverse complement of nucleotides 1756898 to 1757314 of NC_000913.2)
sufS2 also known as csdA (924, 942)	(NP_417290.1; NC_000913.2 nucleotides 2941359 to 2942564)
sufE2 also known as csdE (926, 944)	(NP_417291.1; nucleotides 2942564 to 2943007 of NC_000913.2)
iscA2 also known as erpA (922, 940)	(NP_414698.1; nucleotides 176610 to 176954 of NC_000913.2)
nfu also known as nfuA (923, 941)	(NP_417873.1; nucleotides 3543646 to 3544221 of NC_000913.2)

[0123] Fe uptake and metabolism and/or Fe-S cluster biosynthesis genes, including, but not limited to, those listed in Tables 7, 8 or 9 can potentially be deleted, mutated, expressed, up-regulated, or down-regulated to increase the flux in an Fe-S cluster biosynthesis pathway and improve specific activity of Fe-S cluster requiring proteins such as DHAD. In addition, co-factors can be added to change the activity of polypeptides having Fe-S cluster regulatory activity to increase the flux in an Fe-S cluster biosynthesis pathway and improve DHAD specific activity.

[0124] For example, the genes that increase the flux in an Fe-S cluster biosynthesis pathway can be expressed to improve the activity of DHAD by providing an adequate amount of Fe-S clusters for the apo-enzyme. Any gene, or a combination of them, such as one or more genes listed in Tables 7, 8, or 9, can be cloned and expressed in a pRS411 plasmid as described in Example 4. The resulting constructs, along with the DHAD expression vector pHR81 FBA ilvD(Sm), can then be transformed into wild-type BY4741. As a control, pRS411 without any gene of interest and vector pHR81 FBA ilvD(Sm) are transformed into a wild-type strain. The transformants are selected on agar plates with SD medium without uracil and methionine to maintain both plasmids as described in Example 4. Enzymatic activity for DHAD in the crude extract of different strains from the transformation can be measured. The results can be compared with the specific activity obtained from the control pRS411 without any gene of interest and vector pHR81 FBA ilvD(Sm) transformed into a wild-type strain. An increase in specific

activity indicates a gene that can be used to increase the flux in an Fe-S cluster biosynthesis pathway.

[0125] In addition, strains with deletions in more than one of the genes involved in Fe-S cluster regulatory activity can be created to provide additive effects in improving the enzymes or proteins containing Fe-S cluster(s). For example, double mutants with deletions in both *FRA2* and *GXR3* genes can be used to transform vector pHR81 FBA-IlvD(sm), and the DHAD activity in the crude extract from the transformants can be measured.

[0126] Another alternative is to alter the expression of, *e.g.*, the *PSE1* (SEQ ID NO:777) gene, which encodes a protein involved in the import of Aft1p into the nucleus (Fukunaka, et al, 2003, J. Biological Chem., vol. 278, pp. 50120-50127). Expression of this gene can be accomplished by cloning it in vector pRS411 as described above.

[0127] Thus, provided herein are recombinant host cells that comprise an alteration in the expression of any polypeptide encoded by an Fe uptake and utilization or an Fe-S cluster biosynthesis gene. Encompassed are recombinant host cells that comprise at least one heterologous polynucleotide of any one of the above-referenced Fe-S cluster biosynthesis genes. Also encompassed are recombinant host cells, wherein the host cell comprises at least one deletion, mutation, and/or substitution in an endogenous gene of any one of the above-referenced Fe uptake and utilization or Fe-S cluster biosynthesis genes. Also provided are recombinant host cells that comprise at least one heterologous polynucleotide of any one of the above-referenced Fe uptake and utilization or Fe-S cluster biosynthesis genes, wherein the host cell comprises at least one deletion, mutation, and/or substitution in an endogenous gene of any one of the above-referenced Fe uptake and utilization or Fe-S cluster biosynthesis genes.

[0128] These recombinant host cells can also comprise at least one heterologous Fe-S cluster requiring protein. For example, provided herein is a recombinant host cell comprising at least one heterologous DHAD and at least one heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis. Also provided is a recombinant host cell comprising at least one heterologous DHAD, wherein the host cell comprises at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis. Also provided is a recombinant host cell comprising at least one heterologous DHAD and at least one

heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis, wherein the host cell comprises at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis.

[0129] Host cells that can be used in the present invention include yeast host cells including, but not limited to, *Saccharomyces*, *Schizosaccharomyces, Hansenula*, *Candida*, *Kluyveromyces*, *Yarrowia, Issatchenkia,* and *Pichia*. Bacterial host cells can also be used to create recombinant host cells that comprise at least one heterologous polynucleotide encoding a polypeptide having DHAD activity and at least one heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis. For example, lactic acid bacteria comprising recombinant DHAD and at least one recombinant genetic expression element encoding Fe-S cluster forming proteins are the subject of U.S. Appl. No. 12/569,103, filed Sept. 29, 2009, which is incorporated by reference herein. The present recombinant host cells comprising at least one heterologous polynucleotide encoding a polypeptide having DHAD activity and at least one heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis do not include those lactic acid bacteria described in U.S. Appl. No. 12/569,103, filed Sept. 29, 2009, which is incorporated by reference herein.

[0130] The polypeptide affecting Fe-S cluster biosynthesis can be selected from the group consisting of the Fe uptake and utilization or Fe-S cluster biosynthesis pathway genes in Tables 7, 8 and 9. In one embodiment, the polypeptide affecting Fe-S cluster biosynthesis is encoded by *ARN1, ARN2, ATX1, CCC2, COT1, ENB1, FET3, FET5, FIT1, FIT2, FIT3, FRE1, FRE2, FRE3, FRE4, FRE5, FRE6, FTH1, FTR1, HMX1, SIT1, SMF3, TIS11,VHT1, AFT1, AFT2, AIM1, ARH1, ATM1, BUD32, CAD1, CCC1, CFD1, CIA1, CMK1, CTH1, CTI6, CYC8, DAP1, DRE2, ERV1, ESA1, FET4, FRA1, FRA2, GEF1, GGC1, GRX1, GRX2, GRX4, GRX5, HDA1, IBA57, ISA1, ISA2, ISU1, ISU2, JAC1, MGE1, MRS3, MRS4, MSN5, NAR1, NFS1, NFU1, NHP6a, NHP6b, PSE1, SMF1, SNF1, SNF2, SNF3, SNF4, SSQ1, TIM12, TUP1,* NP_011911.1, *VPS41, YAP5, YFH1, YRA1, ZPR1, iscA^{nif}, nifU, nifS, cysE1, cysE2, iscS, iscU, iscA, hscB, hscA, Fdx, sufS, sufE, cysE3, sufS2, iscA2, Nfu, nfuA, nfuV, nfu, sufA, sufB, sufC, sufD, sufE1, sufS2,* or sufE2. In one embodiment, the polypeptide affecting Fe-S cluster biosynthesis is AFT1, AFT2, PSE1, FRA2, GRX3, or MSN5. In one embodiment, the polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of AFT1, AFT2, PSE1, FRA2, GRX3, MSN5, and combinations thereof. In one embodiment, the polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of AFT1, AFT2, PSE1, FRA2,

MSN5, and combinations thereof. In another embodiment, the polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of AFT1, AFT2, PSE1, FRA2, GRX3, MSN5, and combinations thereof, and the polypeptide affecting Fe-S cluster biosynthesis is encoded by a polynucleotide comprising a plasmid. In some embodiments, DHAD is co-expressed with AFT1, AFT2, PSE1, and combinations thereof. The polypeptide affecting Fe-S cluster biosynthesis may be a constitutive mutant, such as, but not limited to, AFT1 L99A, AFT1 L102A, AFT1 C291F, AFT1 C293F, and combinations thereof. The deletion, mutation, and/or substitution in the endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis can be selected from the group consisting of FRA2, GRX3, MSN5, and combinations thereof.

[0131] The present invention also provides a method for increasing the activity of an Fe-S cluster requiring protein in a recombinant host cell comprising providing a recombinant host cell comprising an Fe-S cluster requiring protein, changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis in the host cell, and growing the recombinant host cell with the changed expression or activity under conditions whereby the activity of the Fe-S cluster requiring protein is increased. Such a method can be used to increase the activity of an endogenous Fe-S cluster requiring protein, or a heterologous Fe-S cluster requiring protein. Such a method can be used to increase the specific activity of a DHAD described herein, or identified by the methods described herein. The increase in the activity of the Fe-S cluster requiring protein can be in an amount selected from greater than about 10%; greater than about 15%; greater than about 20%; greater than about 25%; greater than about 30%; greater than about 35%; greater than about 40%; greater than about 45%; greater than about 50%; greater than about 55%; greater than about 60%; greater than about 65%; greater than about 70%; greater than about 75%; greater than about 80%; greater than about 85%; greater than about 90%; and greater than about 95%. The increase in activity may be greater than about 3 fold, greater than about 5 fold, greater than about 8 fold, or greater than about 10 fold. In embodiments, the activity of the Fe-S cluster requiring protein can be in an amount that is at least about 60% of theoretical, at least about 70% of theoretical, at least about 80% theoretical, or at least about 90% theoretical.

[0132] The present invention can also be used to increase the flux in the Fe-S cluster biosynthesis pathway in a host cell and to identify polypeptides that increase the flux in

an Fe-S cluster biosynthesis pathway in a host cell. In one embodiment a method is provided for increasing the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising providing a recombinant host cell comprising an Fe-S cluster requiring protein and either at least one polypeptide affecting Fe-S cluster biosynthesis, at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis, or a combination of both, and growing the recombinant host cell under conditions whereby the flux in the Fe-S cluster biosynthesis pathway in the host cell is increased. In another embodiment, a method is provided for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising: (a) changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis; (b) measuring the activity of a Fe-S cluster requiring protein; and (c) comparing the activity of the Fe-S cluster requiring protein measured in the presence of the change in expression or activity polypeptide of step (a) to the activity of the Fe-S cluster requiring protein measured in the absence of the change in expression or activity polypeptide of step (a), wherein an increase in the activity of the heterologous Fe-S cluster requiring protein indicates an increase in the flux in said Fe-S cluster biosynthesis pathway. In such methods, the Fe-S cluster requiring protein may be endogenous or heterologous to the host cell.

[0133] The expression or activity of the polypeptide affecting Fe-S cluster biosynthesis can be changed by methods well known in the art, including, but not limited to, deleting, mutating, substituting, expressing, up-regulating, down-regulating, altering the cellular location, altering the state of the protein, and/or adding a cofactor, and combinations thereof. Altering the state of the protein can include, but are not limited to, such alterations as phosphorylation or ubiquitination. Any number of methods described herein or known in the art can be used to measure the activity of the Fe-S cluster requiring protein, depending upon the Fe-S cluster requiring protein chosen. For example, if DHAD is the Fe-S cluster requiring protein, the assay described in the Example 7 can be used to measure the activity of the DHAD to determine if there is an increase in the flux in the Fe-S cluster biosynthesis pathyway of the host cell.

[0134] Isobutanol and Other Products

[0135] Expression of a DHAD in a recombinant host cell, as described herein, provides the transformed, recombinant host cell with dihydroxy-acid dehydratase activity for

conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate or 2,3-dihydroxymethylvalerate to α-ketomethylvalerate. A product that has α-ketoisovalerate or α-ketomethylvalerate as a pathway intermediate may be produced with greater effectiveness in a host cell disclosed herein having the described heterologous DHAD. A list of such products includes, but is not limited to, valine, isoleucine, leucine, pantothenic acid, 2-methyl-1-butanol, 3-methyl-1-butanol, and isobutanol.

[0136] For example, biosynthesis of valine in yeast includes steps of acetolactate conversion to 2,3-dihydroxy-isovalerate by acetohydroxyacid reductoisomerase (ILV5), conversion of 2,3-dihydroxy-isovalerate to α-ketoisovalerate (also called 2-keto-isovalerate) by dihydroxy-acid dehydratase, and conversion of α-ketoisovalerate to valine by branched-chain amino acid transaminase (BAT2) and branched-chain animo acid aminotransferase (BAT1). Biosynthesis of leucine includes the same steps to α-ketoisovalerate, followed by conversion of α-ketoisovalerate to alpha-isopropylmalate by alpha-isopropylmalate synthase (LEU9, LEU4), conversion of alpha-isopropylmalate to beta-isopropylmalate by isopropylmalate isomerase (LEU1), conversion of beta-isopropylmalate to alpha-ketoisocaproate by beta-IPM dehydrogenase (LEU2), and finally conversion of alpha-ketoisocaproate to leucine by branched-chain amino acid transaminase (BAT2) and branched-chain amino acid aminotransferase (BAT1). The bacterial pathway is similar, involving differently named proteins and genes. Increased conversion of 2,3-dihydroxy-isovalerate to α-ketoisovalerate will increase flow in these pathways, particularly if one or more additional enzymes of a pathway is overexpressed. Thus, it is desired for production of valine or leucine to use a strain disclosed herein.

[0137] Biosynthesis of pantothenic acid includes a step performed by DHAD, as well as steps performed by ketopantoate hydroxymethyltransferase and pantothenate synthase. Engineering of expression of these enzymes for enhanced production of pantothenic acid biosynthesis in microorganisms is described in U.S. Patent No. 6,177,264.

[0138] The α-ketoisovalerate product of DHAD is an intermediate in isobutanol biosynthetic pathways disclosed in U.S. Patent Appl. Pub. No. 20070092957 A1, which is incorporated by reference herein. A diagram of disclosed isobutanol biosynthetic pathways is provided in Figure 5. Production of isobutanol in a strain disclosed herein may benefit from increased DHAD activity. As disclosed herein, increased DHAD activity is provided by expression of a DHAD in a host cell, for example, by over-

expressing the DHAD, by modulating the expression or activity of a polypeptide having Fe-S cluster regulatory activity, or a combination of both expression of a DHAD and modulation of the expression or activity of a polypeptide having Fe-S cluster regulatory activity. As described in U.S. Patent Appl. Pub. No. 20070092957 A1, which is incorporated by reference herein, steps in an example isobutanol biosynthetic pathway include conversion of:

[0139] - pyruvate to acetolactate (*see* Fig. 5, pathway step a therein), as catalyzed for example by acetolactate synthase,

[0140] - acetolactate to 2,3-dihydroxyisovalerate (*see* Fig. 5, pathway step b therein) as catalyzed for example by acetohydroxy acid isomeroreductase;

[0141] - 2,3-dihydroxyisovalerate to α-ketoisovalerate (*see* Fig. 5, pathway step c therein) as catalyzed for example by acetohydroxy acid dehydratase, also called dihydroxy-acid dehydratase (DHAD);

[0142] - α-ketoisovalerate to isobutyraldehyde (*see* Fig. 5, pathway step d therein) as catalyzed for example by branched-chain α-keto acid decarboxylase; and

[0143] - isobutyraldehyde to isobutanol (*see* Fig. 5, pathway step e therein) as catalyzed for example by branched-chain alcohol dehydrogenase.

[0144] The substrate to product conversions, and enzymes involved in these reactions, for steps f, g, h, I, j, and k of alternative pathways are described in U.S. Patent Appl. Pub. No. 20070092957 A1, which is incorporated by reference herein.

[0145] Genes that can be used for expression of the pathway step enzymes named above other than the DHADs disclosed herein, as well as those for additional isobutanol pathways, are described in U.S. Patent Appl. Pub. No. 20070092957 A1, which is incorporated by reference herein. Additional genes that may be used can be identified by one skilled in the art through bioinformatics or using methods well-known in the art, such as the various methods described in U.S. Appl. No. 12/569,636, filed Sept. 29, 2009, which is incorporated by reference herein, to isolate homologs. Suitable ketol-acid reductoisomerase (KARI) enzymes are described in U.S. Patent Appl. Pub. Nos. 20080261230 A1, 20090163376, 20100197519, and U.S. Appl. No. 12/893077, all incorporated by reference herein. Examples of KARIs disclosed therein are those from *Vibrio cholerae*, *Pseudomonas aeruginosa* PAO1, and *Pseudomonas fluorescens* PF5.

U.S. Patent Appl. Publ. No. 2009/0269823 and U.S. Prov. Patent Appl. No. 61/290,636, incorporated by reference herein, describe suitable alcohol dehydrogenases.

[0146] Additionally described in U.S. Patent Appl. Pub. No. 20070092957 A1, which is incorporated by reference herein, are construction of chimeric genes and genetic engineering of bacteria and yeast for isobutanol production using the disclosed biosynthetic pathways.

[0147] Additional modifications

[0148]

[0149] Examples of additional modifications that may be useful in cells provided herein include modifications to reduce glycerol-3-phosphate dehydrogenase activity and/or disruption in at least one gene encoding a polypeptide having pyruvate decarboxylase activity or a disruption in at least one gene encoding a regulatory element controlling pyruvate decarboxylase gene expression as described in U.S. Patent Appl. Pub. No. 20090305363 (incorporated herein by reference), modifications to a host cell that provide for increased carbon flux through an Entner-Doudoroff Pathway or reducing equivalents balance as described in U.S. Patent Appl. Pub. No. 20100120105 (incorporated herein by reference). Other modifications include integration of at least one polynucleotide encoding a polypeptide that catalyzes a step in a pyruvate-utilizing biosynthetic pathway described in U.S. Prov. Appl. No. 61/380563 (incorporated herein by reference). Additional modifications that may be suitable are described in U.S, Appl. Serial No. 12/893089. Additionally, host cells comprising a heterologous polynucleotide encoding a polypeptide with phosphoketolase activity and host cells comprising a heterologous polynucleotide encoding a polypeptide with phosphotransacetylase activity are described in US Provisional Patent Application No. 61/356379. Growth for production

[0150] Recombinant host cells disclosed herein are grown in fermentation media which contains suitable carbon substrates. Suitable carbon substrates may include, but are not limited to, monosaccharides such as glucose, fructose, oligosaccharides such as lactose maltose, galactose, 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. Other carbon substrates may include ethanol, lactate, succinate, or glycerol.

[0151] Additionally the carbon substrate may also be one-carbon substrates such as carbon dioxide, or methanol for which metabolic conversion into key biochemical intermediates has been demonstrated. Two-carbon substrates such as ethanol may also suitable. 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 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.

[0152] Although it is contemplated that all of the above mentioned carbon substrates and mixtures thereof are suitable in the present invention, in some embodiments, the carbon substrates are glucose, fructose, and sucrose, or mixtures of these with C5 sugars such as xylose and/or arabinose for yeasts cells modified to use C5 sugars. Sucrose may be derived from renewable sugar sources such as sugar cane, sugar beets, cassava, sweet sorghum, and mixtures thereof. Glucose and dextrose may be derived from renewable grain sources through saccharification of starch based feedstocks including grains such as corn, wheat, rye, barley, oats, and mixtures thereof. In addition, fermentable sugars may be derived from renewable cellulosic or lignocellulosic biomass through processes of pretreatment and saccharification, as described, for example, in co-owned and co-pending U.S. Patent Appl. Pub. No. 20070031918 A1, 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. 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 may comprise a mixture of corn cobs and corn stover, or a mixture of grass and leaves. Biomass includes, but is not limited to, 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, 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, animal manure, and mixtures thereof.

[0153] In addition to an appropriate carbon source, growth 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 comprising a Fe-S cluster requiring protein such as, for example, DHAD.

[0154] Culture Conditions

[0155] Typically cells are grown at a temperature in the range of about 20 °C to about 40 °C in an appropriate medium. Suitable growth media in the present invention are common commercially prepared media such as Luria Bertani (LB) broth, Sabouraud Dextrose (SD) broth, Yeast Medium (YM) broth, or broth that includes yeast nitrogen base, ammonium sulfate, and dextrose (as the carbon/energy source) or YPD Medium, a blend of peptone, yeast extract, and dextrose in optimal proportions for growing most *Saccharomyces cerevisiae* strains. 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 indirectly, *e.g.*, cyclic adenosine 2':3'-monophosphate, may also be incorporated into the growth medium.

[0156] Suitable pH ranges for the growth are between about pH 5.0 to about pH 9.0. In one embodiment, about pH 6.0 to about pH 8.0 is used for the initial condition. Suitable pH ranges for the fermentation of yeast are typically between about pH 3.0 to about pH 9.0. In one embodiment, about pH 5.0 to about pH 8.0 is used for the initial condition. Suitable pH ranges for the fermentation of other microorganisms are between about pH 3.0 to about pH 7.5. In one embodiment, about pH 4.5 to about pH 6.5 is used for the initial condition.

[0157] Growth may be performed under aerobic or anaerobic conditions. In one embodiment, anaerobic or microaerobic conditions are used for growth.

[0158] Industrial Batch and Continuous Fermentations

[0159] Isobutanol, or other products, may be produced using 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 artificial alterations during the fermentation. 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 the metabolism of the cells and where it is desirable to have limited amounts of substrate in the media. Batch and 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.

[0160] Isobutanol, or other products, may also be produced using 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 processing. Continuous fermentation generally maintains the cultures at a constant high density where cells are primarily in log phase growth. Continuous fermentation allows for the modulation of one factor or any number of factors that affect cell growth or end product concentration. Methods of modulating nutrients and growth factors for continuous fermentation processes as well as techniques for maximizing the rate of product formation are well known in the art of industrial microbiology and a variety of methods are detailed by Brock, *supra*.

[0161] It is contemplated that the production of isobutanol, or other products, may be practiced using batch, fed-batch or continuous processes and that any known mode 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 isobutanol production.

[0162] Methods for Isobutanol Isolation from the Fermentation Medium

[0163] Bioproduced isobutanol may be isolated from the fermentation medium using methods known in the art for ABE fermentations (*see, e.g.*, Durre, *Appl. Microbiol. Biotechnol. 49*:639-648 (1998), Groot *et al.*, *Process. Biochem. 27*:61-75 (1992), and

references therein). For example, solids may be removed from the fermentation medium by centrifugation, filtration, decantation, or the like. Then, the isobutanol may be isolated from the fermentation medium using methods such as distillation, azeotropic distillation, liquid-liquid extraction, adsorption, gas stripping, membrane evaporation, or pervaporation.

[0164] Because isobutanol forms a low boiling point, azeotropic mixture with water, distillation can be used to separate the mixture up to its azeotropic composition. Distillation may be used in combination with another separation method to obtain separation around the azeotrope. Methods that may be used in combination with distillation to isolate and purify butanol include, but are not limited to, decantation, liquid-liquid extraction, adsorption, and membrane-based techniques. Additionally, butanol may be isolated using azeotropic distillation using an entrainer (*see, e.g.*, Doherty and Malone, *Conceptual Design of Distillation Systems*, McGraw Hill, New York, 2001).

[0165] The butanol-water mixture forms a heterogeneous azeotrope so that distillation may be used in combination with decantation to isolate and purify the isobutanol. In this method, the isobutanol containing fermentation broth is distilled to near the azeotropic composition. Then, the azeotropic mixture is condensed, and the isobutanol is separated from the fermentation medium by decantation. The decanted aqueous phase may be returned to the first distillation column as reflux. The isobutanol-rich decanted organic phase may be further purified by distillation in a second distillation column.

[0166] The isobutanol may also be isolated from the fermentation medium using liquid-liquid extraction in combination with distillation. In this method, the isobutanol is extracted from the fermentation broth using liquid-liquid extraction with a suitable solvent. The isobutanol-containing organic phase is then distilled to separate the butanol from the solvent.

[0167] Distillation in combination with adsorption may also be used to isolate isobutanol from the fermentation medium. In this method, the fermentation broth containing the isobutanol is distilled to near the azeotropic composition and then the remaining water is removed by use of an adsorbent, such as molecular sieves (Aden *et al. Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis for Corn Stover*, Report NREL/TP-510-32438, National Renewable Energy Laboratory, June 2002).

[0168] Additionally, distillation in combination with pervaporation may be used to isolate and purify the isobutanol from the fermentation medium. In this method, the fermentation broth containing the isobutanol is distilled to near the azeotropic composition, and then the remaining water is removed by pervaporation through a hydrophilic membrane (Guo *et al.*, *J. Membr. Sci.* 245, 199-210 (2004)).

[0169] Embodiments of the Inventions

[0170] Embodiment 1 (E1). A recombinant host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity wherein said at least one heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated.

[0171] E2. A recombinant host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity wherein said at least one heterologous polynucleotide is integrated at least once in the recombinant host cell DNA.

[0172] E3. A recombinant host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity, wherein said host cell comprises at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis.

[0173] E4. A recombinant host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity and at least one heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis.

[0174] E5. The recombinant host cell of any one of embodiments E3-E4, wherein said heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of the genes in Tables 8 and 9.

[0175] E6. The recombinant host cell of any one of embodiments E3-E4, wherein said heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of the genes in Table 7.

[0176] E7. The recombinant host cell of embodiment E5 or E6, wherein said heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis is

selected from the group consisting of AFT1, AFT2, PSE1, FRA2, GRX3, MSN5. and combinations thereof.

[0177] E8. The recombinant host cell of embodiment E7, wherein said polypeptide is encoded by a polynucleotide that is constitutive mutant.

[0178] E9. The recombinant host cell of embodiment E8, wherein said constitutive mutant is selected from the group consisting of AFT1 L99A, AFT1 L102A, AFT1 C291F, AFT1 C293F, and combinations thereof.

[0179] E10. The recombinant host cell of embodiment E7, wherein said polypeptide affecting Fe-S cluster biosynthesis is encoded by a polynucleotide comprising a high copy number plasmid or a plasmid with a copy number that can be regulated.

[0180] E11. The recombinant host cell of embodiment E7, wherein said polypeptide affecting Fe-S cluster biosynthesis is encoded by a polynucleotide integrated at least once in the recombinant host cell DNA.

[0181] E12. The recombinant host cell of embodiment E3, wherein the at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of FRA2, GRX3, MSN5 , and combinations thereof.

[0182] E13. The recombinant host cell of embodiment E4, wherein the at least one heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of AFT1, AFT2, PSE1, and combinations thereof.

[0183] E14. The recombinant host cell of any one of embodiments E3-E13, wherein said at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity is expressed in multiple copies.

[0184] E15. The recombinant host cell of embodiment E14, wherein said at least one heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated.

[0185] E16. The recombinant host cell of embodiment E14, wherein said at least one heterologous polynucleotide is integrated at least once in the recombinant host cell DNA.

[0186] E17. The recombinant host cell of any one of embodiments E3-E16, wherein said Fe-S cluster biosynthesis is increased compared to a recombinant host cell having endogenous Fe-S cluster biosynthesis.

[0187] E18. The recombinant host cell of any one of embodiments E1-E17, wherein said host cell is a yeast host cell.

[0188] E19. The recombinant host cell of embodiment E18, wherein said yeast host cell is selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Hansenula*, *Candida*, *Kluyveromyces*, *Yarrowia*, *Issatchenkia* and *Pichia.*

[0189] E20. The recombinant host cell of any one of embodiments E1-E19, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in the cytosol of the host cell.

[0190] E21. The recombinant host cell of any one of embodiments E1-E20, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of $< 10^{-5}$ wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the *Streptococcus mutans* DHAD enzyme corresponding to SEQ ID NO:168.

[0191] E22. The recombinant host cell of any one of embodiments E1-E21, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence with at least about 90% identity to SEQ ID NO: 168 or SEQ ID NO: 232.

[0192] E23. The recombinant host cell of any one of embodiments E1-E22, wherein said polypeptide having dihydroxy-acid dehydratase activity has a specific activity selected from the group consisting of:

a. greater than about 5-fold with respect to the control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity;
b. greater than about 8-fold with respect to the control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity; and
c. greater than about 10-fold with respect to the control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity.

[0193] E24. The recombinant host cell of any one of embodiments E1-E22, wherein said polypeptide having dihydroxy-acid dehydratase activity has a specific activity selected from the group consisting of:

a. greater than about 0.25 U/mg;

b. greater than about 0.3 U/mg;

c. greater than about 0.5 U/mg;

d. greater than about 1.0 U/mg;

e. greater than about 1.5 U/mg;

f. greater than about 2.0 U/mg;

g. greater than about 3.0 U/mg;

h. greater than about 4.0 U/mg;

i. greater than about 5.0 U/mg;

j. greater than about 6.0 U/mg;

k. greater than about 7.0 U/mg;

l. greater than about 8.0 U/mg;

m. greater than about 9.0 U/mg;

n. greater than about 10.0 U/mg;

o. greater than about 20.0 U/mg; and

p. greater than about 50.0 U/mg.

[0194] E25. The recombinant host cell of any one of embodiments E1-E24, wherein said recombinant host cell produces isobutanol.

[0195] E26. The recombinant host cell of embodiment E25, wherein said recombinant host cell comprises an isobutanol biosynthetic pathway.

[0196] E27. A method of making a product comprising:

a. providing the recombinant host cell of any one of embodiments E1-E24; and

b. contacting the recombinant host cell of (a) with a fermentable carbon substrate in a fermentation medium under conditions wherein said product is produced;

wherein the product is selected from the group consisting of branched chain amino acids, pantothenic acid, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutanol, and combinations thereof.

[0197] E28. A method of making isobutanol comprising:

a. providing the recombinant host cell of any one of embodiments E1-E24;

b. contacting the recombinant host cell of (a) with a fermentable carbon substrate in a fermentation medium under conditions wherein isobutanol is produced.

[0198] E29. A method for the conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate comprising:

a. providing the recombinant host of any one of embodiments E1-E24;

b. growing the recombinant host cell of (a) under conditions where the 2,3-dihydroxyisovalerate is converted to α-ketoisovalerate,

wherein 2,3-dihydroxyisovalerate is converted to α-ketoisovalerate.

[0199] E30. A method for increasing the specific activity of a heterologous polypeptide having dihydroxy-acid dehydratase activity in a recombinant host cell comprising:

a. providing a recombinant host cell of any one of embodiments E1-E24; and

b. growing the recombinant host cell of (a) under conditions whereby the heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in functional form having a specific activity greater than the same host cell lacking said heterologous polypeptide.

[0200] E31. A method for increasing the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising:

a. providing a recombinant host cell of any one of embodiments E3-E24; and

b. growing the recombinant host cell of (a) under conditions whereby the flux in the Fe-S cluster biosynthesis pathway in the host cell is increased.

[0201] E32. A method of increasing the activity of an Fe-S cluster requiring protein in a recombinant host cell comprising:

a. providing a recombinant host cell comprising an Fe-S cluster requiring protein;

b. changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis in said host cell; and

c. growing the recombinant host cell of (b) under conditions whereby the activity of the Fe-S cluster requiring protein is increased.

[0202] E33. The method of embodiment E32, wherein said increase in activity is an amount selected from the group consisting of:

a. greater than about 10%;

b. greater than about 20%;

c. greater than about 30%;

d. greater than about 40%;

e. greater than about 50%;

f. greater than about 60%;

g. greater than about 70%;

h. greater than about 80%;

i. greater than about 90%; and

j. greater than about 95%.

[0203] E34. The method of embodiment E32, wherein said increase in activity is an amount selected from the group consisting of:

a. greater than about 5 fold;

b. greater than about 8 fold;

c. greater than about 10 fold.

[0204] E35. A method for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising:

a. changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis;

b. measuring the activity of a heterologous Fe-S cluster requiring protein; and

c. comparing the activity of the heterologous Fe-S cluster requiring protein measured in the presence of the changed expression or activity of a polypeptide of step (a) to the activity of the heterologous Fe-S cluster requiring protein measured in the absence of the changed expression or activity of a polypeptide of step (a),

wherein an increase in the activity of the heterologous Fe-S cluster requiring protein indicates an increase in the flux in said Fe-S cluster biosynthesis pathway.

[0205] E36. A method for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising:

a. changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis;

b. measuring the activity of a polypeptide having dihydroxy-acid dehydratase activity; and

c. comparing the activity of the polypeptide having dihydroxy-acid dehydratase activity measured in the presence of the change in expression or activity of a polypeptide of step (a) to the activity of the polypeptide having dihydroxy-acid dehydratase activity measured in the absence of the change in expression or activity of a polypeptide of step (a),

wherein an increase in the activity of the polypeptide having dihydroxy-acid dehydratase activity indicates an increase in the flux in said Fe-S cluster biosynthesis pathway.

[0206] E37. The method of any one of embodiments E30-E36, wherein said changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis comprises deleting, mutating, substituting, expressing, up-regulating, down-regulating, altering the cellular location, altering the state of the protein, and/or adding a cofactor.

[0207] E38. The method of any one of embodiments E32-E37, wherein the Fe-S cluster requiring protein has dihydroxy-acid dehydratase activity and wherein said Fe-S cluster requiring protein having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of $< 10^{-5}$ wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the *Streptococcus mutans* DHAD enzyme corresponding to SEQ ID NO:168.

[0208] E39. The method of any one of embodiments E32-E38, wherein said polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of the genes in Tables 7, 8 and 9.

[0209] E40. A recombinant host cell comprising at least one polynucleotide encoding a polypeptide identified by the methods of any one of embodiments E35-E37.

[0210] E41. The recombinant host cell of embodiment E40, wherein said host cell further comprises at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity.

[0211] E42. The recombinant host cell of embodiment E41, wherein said heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity is expressed in multiple copies.

[0212] E43. The recombinant host cell of embodiment E41, wherein said heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated.

[0213] E44. The recombinant host cell of embodiment E41, wherein said heterologous polynucleotide is integrated at least once in the recombinant host cell DNA.

[0214] E45. The method of embodiment E35 or E36, wherein said host cell is a yeast host cell.

[0215] E46. The method of embodiment E45, wherein said yeast host cell is selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Hansenula*, *Candida*, *Kluyveromyces*, *Yarrowia*, *Issatchenkia,* and *Pichia*.

[0216] E47. The method of any one of embodiments E28-E39, wherein said host cell is a yeast host cell.

[0217] E48. The method of embodiment E47, wherein said yeast host cell is selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Hansenula*, *Candida*, *Kluyveromyces*, *Yarrowia*, *Issatchenkia,* and *Pichia*.

[0218] E49. The recombinant host cell of any one of embodiments E40-E44, wherein said recombinant host cell is a yeast host cell.

[0219] E50. The recombinant host cell of embodiment E49, wherein said yeast host cell is selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Hansenula*, *Candida*, *Kluyveromyces*, *Yarrowia*, *Issatchenkia,* and *Pichia*.

[0220] E51. The recombinant host cell of any one of embodiments E40-E44 or E49-E50, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in the cytosol of the host cell.

[0221] E52. The recombinant host cell of any one of embodiments E40-E44 or E49-E50, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of $< 10^{-5}$ wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the *Streptococcus mutans* DHAD enzyme corresponding to SEQ ID NO:168.

[0222] E53. The recombinant host cell of any one of embodiments E40-E44 or E49-E50, wherein said recombinant host cell produces a product selected from the group consisting of branched chain amino acids, pantothenic acid, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutanol, and combinations thereof.

[0223] E54. The recombinant host cell of embodiment E53, wherein said recombinant host cell produces isobutanol.

[0224] E55. The recombinant host cell of embodiment E54, wherein said recombinant host cell comprises an isobutanol biosynthetic pathway.

Examples

[0225] The meaning of abbreviations used is as follows: "min" means minute(s), "h" means hour(s), "sec" means second(s), "µl" means microliter(s), "ml" means milliliter(s), "L" means liter(s), "nm" means nanometer(s), "mm" means millimeter(s), "cm" means

centimeter(s), "μm" means micrometer(s), "mM" means millimolar, "M" means molar, "mmol" means millimole(s), "μmole" means micromole(s), "g" means gram(s), "μg" means microgram(s), "mg" means milligram(s), "rpm" means revolutions per minute, "w/v" means weight/volume, "OD" means optical density, and "OD_{600}" means optical density measured at a wavelength of 600 nm.

[0226] GENERAL METHODS:

[0227] Standard recombinant DNA and molecular cloning techniques used 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, N.Y., 1989, by T. J. Silhavy, M. L. Bennan, and L. W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984, and by Ausubel, F. M. *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience, N.Y., 1987.

[0228] Materials and methods suitable for the maintenance and growth of bacterial cultures are also well known in the art. Techniques suitable for use in the following Examples may be found 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 Microbiology*, Second Edition, Sinauer Associates, Inc., Sunderland, MA, 1989. All reagents, restriction enzymes and materials used 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, MO), unless otherwise specified.

[0229] **Example 1. Over-expression of DHAD protein encoded by the *ilvD* gene from *S. mutans* using a plasmid-based system in yeast cytosol.**

[0230] Over-expression of a recombinant polynucleotide can be accomplished by increasing the copy number of a plasmid comprising the recombinant polynucleotide. To over-express the DHAD protein in yeast, an inducible vector was constructed. The pHR81 vector contains a *Ura3* marker as well as a *LEU* marker with a defective promoter

(*see* U.S. Patent Appl. Pub. No. 2007/0092957, which is incorporated by reference herein). When the yeast synthetic dropout (SD; also known as complete minimal media; Teknova) growth medium is switched from SD minus uracil to SD minus leucine, the copy number of the pHR81 plasmid increases, resulting in much higher level of expression of the recombinant polynucleotide. The pHR81 vector backbone was derived from pLH472 JEG4y (SEQ ID NO: 921) and was prepared by digesting the pLH472 JEG4y vector with SpeI and SacII.

[0231] For over-expression of a DHAD protein, the DHAD gene *ilvD* from *S. mutans* (SEQ ID NO:167) was used (*see* U.S. Published Patent Appl. No. US2009-0305363A1, which is incorporated by reference herein). This gene has been cloned under the control of the FBA promoter in vector pRS423 FBA ilvD Strep-lumio (*see* U.S. Published Patent Appl. No. US2009-0305363A1, which is incorporated by reference herein). The region containing the FBA promoter, the *ilvD* gene, and FBA terminator cassette was amplified with primer set FBAp-F(NheI) and FBAt-R(SacII) (SEQ ID NOs: 915 and 916) and cloned into the pHR81 vector. The resulting expression vector was designated as pHR81 FBA-IlvD(Sm) (SEQ ID NO: 917; Figure 1A).

[0232] To over express the *S. mutans* DHAD protein, the expression vector pHR81 FBA-IlvD(Sm) was transformed into wild-type yeast strain BY4741. Transformants were selected on agar plates with SD minus uracil. For over-expression, yeast strains containing the plasmid were initially grown at 30° C in SD liquid medium minus uracil. A fresh overnight culture (5 ml) was then transferred to a 125 ml flask containing 75 ml of SD medium minus leucine. As a control, another 5 ml of fresh overnight culture was transferred into a flask containing 75 ml of SD minus uracil. The cultures were incubated overnight before harvesting by centrifugation. The DHAD activity was measured in crude extracts of these samples using the assay described in Example 7.

[0233] The DHAD specific activity obtained in the crude extract in the control samples grown in SD minus uracil was in the range of 0.2 to 0.3 U mg^{-1}. The average specific activity obtained from strains grown in the SD medium minus leucine, however, was 1.6 U mg^{-1}, much higher (~5 to 8-fold higher) than the activity from the control samples. DHAD requires Fe-S cluster for its function, and it was not previously known if the native yeast Fe-S cluster biosynthesis pathway could accommodate an over-expressed Fe-S cluster requiring protein in yeast cytosol. In a previous screening experiment using a

non-inducible, low-copy number vector, the DHAD from *S. mutans* could be recombinantly expressed in yeast cytosol with a specific activity in the range of 0.1 to 0.2 U mg^{-1} in the crude extract (*see* U.S. Patent Appl. No. 12/569,636, filed on Sept. 29, 2009, which is incorporated by reference herein). Thus, in one embodiment, over-expression of a Fe-S cluster requiring protein, such as DHAD, in yeast using a high-copy number vector provides increased specific activity, wherein the specific activity is increased by at least about 5-fold to at least about 8-fold.

[0234] Example 2. Over-expression of DHAD protein encoded by the *ilvD* gene from *S. mutans* through chromosomal integration.

[0235] An alternate way to increase the expression of a gene in yeast is to integrate multiple copies of the gene of interest into the host cell's chromosome. To integrate the *ilvD* gene from *S. mutans* (SEQ ID NO:167) into a yeast chromosome, integration vector pZK-Delta(s)-Leu2-FBA-ilvD(Sm)-FBAt (SEQ ID NO: 918; Figure 1B) was constructed. The integration vector backbone was derived from pSuperscript (Stratagene, La Jolla, CA). The *S. mutans ilvD* gene (nucleotides 1306-3018 of the complement strand) was cloned into the integration vector under the control of the FBA promoter (nucleotides 3026-4023 of the complement strand) so that the *ilvD* gene would be flanked by a yeast delta sequence (nucleotides 118-267 and 5061-5760 of the complement strand). *S. cerevisiae* contains more than 200 yeast delta sequences (Kim J M *et al.* Genome Res. 1998; 8:464-478). These delta sequences are targets for multiple integrations. The integration vector was also engineered to contain the defective *LEU2* marker (nucleotides 4100-5191 of the complement strand) for selection of transformed strains with multiple integration events.

[0236] For integration, the vector DNA was linearized with AscI and AatII digestion to generate delta sequence flanked strands of vector DNA comprising the *ilvD* gene, which were then transformed into the yeast strain BY4741. Transformants were selected on SD agar medium minus leucine. These transformants were then grown on SD liquid medium minus leucine at 30° C, and the cultures were harvested and analyzed for DHAD activity. The specific activity of DHAD obtained in the crude extract ranged from 0.7 to 1.2 U

mg^{-1}. This specific activity was about 3- to 6-fold higher than that found in BY4741 strains transformed with an *ilvD* gene-containing plasmid without over-expression

[0237] Example 3. Improvement of specific activity of DHAD in yeast deletion strains

[0238] Although the over-expression strains described in Examples 1 and 2 had a high level of activity, not all of the DHAD protein expressed was active. For example, the over-expressed DHAD protein accounted for approximately 5 to 10% of the total cell protein, while yielding a specific activity of from about 0.7 to 1.6 U mg^{-1}. Given that the specific activity of the purified DHAD enzyme from *S. mutans* is 100 U mg^{-1}, expression of DHAD at 10% of total cell protein would be expected to yield a specific activity upwards of 5 to 10 U mg^{-1}. Although not wishing to be bound by one theory, the difference between the expected and observed specific activity was likely a result of insufficient Fe-S cluster loading. Thus, increasing Fe-S cluster loading by further manipulating the over-expression strains could be used to increase the specific activity of DHAD.

[0239] In order to improve the specific activity, yeast strains with deletions in genes involved in iron metabolism and Fe-S cluster sensing were used to investigate their effects on DHAD specific activity. These strains (BY4741 background) were purchased from Open Biosystem (Huntsville, AL) and are listed in Table 10. As described in Example 1, the high copy number plasmid pHR81 FBA-IlvD(Sm) was transformed into these strains, and DHAD over-expression was induced by changing the growth medium to SD minus leucine. Crude extracts from cultures were prepared and assayed for DHAD activity. Results are shown in Table 10.

Table 10. Effects of deletions of genes involved in Fe metabolism.

Genes	Function	Specific Activity (U/mg)
WT		1.69 ± 0.02
Δ*isu1*	scaffold protein for Fe-S cluster assembling	1.31 ± 0.56
Δ*fra2*	repressor component for Aft1p	3.41 ± 0.24
Δ*sin4*	regulatory protein	1.65 ± 0.20
Δ*mtm1*	protein involved in metal metabolism	0.54 ± 0.12
Δ*fra1*	regulatory protein	0.97 ± 0.05
Δ*grx3*	glutaredoxins	5.45 ± 0.14

Δ*aft1*	global Fe regulator	0.23 ± 0.05
Δ*aft2*	paralogue to Aft1p	1.11 ± 0.38
Δ*msn5*	nuclear protein exporter	1.59 ± 0.10
Δ*fet3*	ferrous iron uptake; multi-copper oxidase	0.54 ± 0.09
Δ*ftr1*	ferrous iron uptake; permease	0.76 ± 0.03
Δ*ccc2*	copper transporter (for Fet3p)	1.23 ± 0.17
Δ*gef1*	copper transporter/loading for Fet3p	1.70 ± 0.10
Δ*fet4*	Low-affinity Fe(II) transporter	1.07 ± 0.02
Δ*smf1*	Low-affinity Fe(II) transporter	1.78 ± 0.12
Δ*mrs3*	mitochondrial iron transporter	1.51 ± 0.13
Δ*mrs4*	mitochondrial iron transporter	0.85 ± 0.16
Δ*cth2*	targeted mRNA binding and degradation	1.28 ± 0.40
Δ*cth1*	targeted mRNA binding and degradation	1.44 ± 0.30

[0240] Surprisingly, DHAD specific activity in the crude extract in strains with a deletion in either the *FRA2* or the *GRX3* gene increased by 2- to 3-fold, which was unexpected as many of the deletions tested did not increase DHAD specific activity. It has been shown that cytosolic iron sulfur assembly (CIA) machinery in yeast is responsible for assembly of Fe-S clusters for cytosolic proteins such as isopropylmalate isomerase (Leu1). Previous results indicate that this CIA machinery is independent from the iron sensing system involving Aft1 and a Grx3/Grx4-Fra2 heterodimer as the repressor (Rutherford *et al*, *J Biol Chem. 280*:10135-10140 (2005)).

[0241] Another unexpected finding is the effect of a *Grx3* deletion on DHAD activity. It has been shown that Grx3 and Grx4 are equivalent in function. While double mutations in both *GRX3* and *GRX4* genes resulted in drastic activation of the Fe regulon, mutation in Grx4 alone confers minimal phenotype (Pujol-Carrion, *et al*, *J Cell Sci. 119*:4554-4564 (2006); Ojeda, *et al*, *J Biol Chem. 281*:17661-17669 (2006).). As shown in Table 10 above, *GRX3* deletion alone leads to significant improvement in DHAD specific activity.

[0242] Thus, these results demonstrate that modulating genes involved in iron metabolism can increase the activity of an Fe-S cluster requiring protein such as DHAD when expressed in yeast cytosol. As outlined in Figure 10, the effect of deletions of the *FRA2* and *GRX3* genes on DHAD specific activity could result from, *e.g.*, activation of transcription of one or more of the genes in the iron regulon via the global regulator

Aft1p. Although not wishing to be bound by any one theory, activation of such genes could lead to an increase in iron uptake and an increase in cytoplasmic Fe-S cluster biosynthesis, leading to higher Fe-S cluster loading of the protein (Figure 10). Demonstration of increased Fe-S cluster loading is described in Example 11.

[0243] Example 4. Effect of expression of Aft1p and its mutants on DHAD specific activity.

[0244] As described in Example 3 and outlined in Figure 10, Fra2, Grx3, and Grx4 are repressors that regulate the function of Aft1p (Kumánovics, *et al.*, *J. Biol. Chem. 283*:10276-10286 (2008)). Aft1p is a global regulator of iron. Activation of genes involved in iron uptake and metabolism requires the nuclear localization of Aft1p. Expression of Aft1 constitutive mutants or an increase in the expression of wild-type Aft1p, could lead to the activation of the Fe regulon in a wild-type strain or in an *AFT1* deletion strain (Yamaguchi-Iwai, *et al*, *EMBO J. 14*:1231-1239 (1995); Yamaguchi-Iwai, *et al*, *J. Biol. Chem. 277*:18914-18918 (2002); Kaplan, *et al*, *Chem. Rev.109*:4536-4552(2009)). Based on the novel findings described in Example 3, it is possible that expression of Aft1p protein and its constitutive mutants may improve the active fraction of the DHAD enzyme which requires Fe-S clusters for its activity.

[0245] To examine this possibility, the wild-type *AFT1* gene and its constitutive mutants were cloned using a centromere vector pRS411 (ATCC® Number: 87538; SEQ ID NO: 919). This vector has an ampicillin selection marker for growth in *E. coli* and a methionine nutritional marker for selection in yeast. The wild-type *AFT1* gene, including its own promoter and terminator, can be cloned between the KpnI and SacI sites, resulting in the construct pRS411-Aft1+flanking (SEQ ID NO: 920; Figure 2). A similar strategy can be used to clone genes that encode Aft1 constitutive mutants. The Aft1 constitutive mutants with substitutions at amino acids L99 to A and C291 to F (with respect to SEQ ID NO: 703) were first examined. The pRS411 constructs with genes encoding the wild-type *AFT1* gene or constitutive mutants were transformed, along with the expression vector pHR81 FBA IlvD(Sm), into the wild-type yeast strain BY4741 or a yeast strain with a deletion in *AFT1*, *GRX3*, or *FRA2*. Transformants were selected on agar plates with SD medium minus methionine and uracil. Transformed strains were grown in SD

medium minus methionine and leucine to over-express the DHAD protein in the presence of these genes or mutants. The DHAD activity in the crude extract of these cultures were measured.

[0246] Results of expression of wild-type Aft1p, Aft1p(C291F), and Aft1p(L99A) are shown in Table 11. A moderate increase in DHAD specific activity was observed with Aft1p (C291F) as compared to wild-type Aft1p. A much higher increase in DHAD activity was observed with Aft1p(L99A). The specific activity of DHAD in yeast expressing Aft1p(L99A) was similar to the specific activity obtained in the GRX3 deletion strain (*see* Table 10).

Table 11. Effects of expression of Aft1p and its mutants on the activity of DHAD from *S. mutans* in Δ*aft1* strain.

Plasmids	Specific Activity (U/mg)
pHR81-FBA-ilvD(Sm) + pRS411-Aft1	2.60 ± 0.52
pHR81-FBA-ilvD(Sm) + pRS411-Aft1(C291L)	3.79 ± 0.23
pHR81-FBA-ilvD(Sm) + pRS411-Aft1(L99A)	5.41 ± 0.41

[0247] **Example 5. Increase in cytosolic DHAD specific activity in a CCC1 deletion strain.**

[0248] The exact mechanism of increasing Fe-S cluster biosynthesis capability for cytosolic DHAD protein is unknown. Based on the findings with FRA2 and GRX3 deletion strains (Example 3) and with expression of Aft1p mutants (Example 4), increasing the availability of the Fe content in the cytosol may also improve the DHAD specific activity. CCC1 deletion has been shown to increase the Fe content of the cytosol (Li L, *et al*, *J Biol Chem. 276*:29515-29519 (2001)). To test this hypothesis, the CCC1 deletion strain of BY4741 was transformed with plasmid pHR81 FBA-IlvD(Sm) as described in Example 1. The crude extracts of cells with the plasmid were assayed for DHAD activity. Table 13 shows the results of the experiment. When the CCC1 deletion strain with the DHAD plasmid was grown in SD medium lacking uracil, an increase in DHAD specific activity was found as compared to the wild-type cells with the same plasmid. When extra Fe was added, a further increase in DHAD was observed in the

CCC1 deletion strain. Addition of Fe showed no effect on DHAD specific activity in the wild-type cells. To achieve an over expression of the DHAD protein, strains were grown in SD medium lacking leucine (Example 1). Under these conditions, an increase in DHAD specific activity was detected.

Table 13. Expression of DHAD from *S. mutans* in the BY4741(Δ*ccc1*) strain.

Strains	Growth conditions	No extra Fe	100 uM Fe
Wild-type	-Ura	0.37 ± 0.03	0.46 ± 0.04
Δ*ccc1*	-Ura	0.83 ± 0.04	1.24 ± 0.03
Wild-type	-Leu	1.60 ± 0.17	1.83 ± 0.31
Δ*ccc1*	-Leu	2.53 ± 0.29	2.7 ± 1.07

[0249] Example 6. Improvement of specific activity of DHAD from *L. lactis* expressed in yeast.

[0250] Examples 1-5 used the DHAD enzyme from *S. mutans* to identify novel ways to increase the specific activity of DHAD when expressed in yeast. In this example, we investigated the application of these methods to improve the specific activity of the DHAD enzyme from *L. lactis* (SEQ ID NO: 958). The *IlvD* gene from *L. lactis* (SEQ ID NO: 959) was cloned into the pHR81 vector under the control of the FBA promoter (Figure 11). The resulting construct pHR81 FBA-IlvD(Ll)-ADHt (Figure 11; SEQ ID NO: 960) was transformed into strains with a deletion in either the *FRA2* or *GRX3* gene. To study the effect of the constitutive mutant Aft1p(L99A) on DHAD from *L. lactis*, pHR81 FBA-IlvD(Ll)-ADHt was co-transformed into yeast host along with vector pRS411-Aft1(L99A) (*see* Example 4). To over-express the *IlvD* gene, transformants were grown in yeast synthetic drop-out medium lacking leucine or lacking both leucine and methionine, depending on the strains. Enzymatic assay results are summarized in Table 14. Deletions in *FRA2* and *GRX3* genes increased the specific activity of the DHAD from *L. lactis* when expressed in yeast. In addition, expression of the Aft1 constitutive mutant L99A similarly increased the specific activity of the DHAD from *L. lactis*.

Table 14. Over-expression of bacterial DHAD from *L. lactis* in *S. cerevisiae*.

Strains	Specific Activity (U/mg)
Wild-type	0.23 ± 0.04
Δ*aft1+Aft1(L99A)*	0.95 ± 0.31
Δ*fra2*	0.72 ± 0.04
Δ*grx3*	0.96 ± 0.05

[0251] Example 7. Determining the Specific Activity of DHAD. (Assay Method)

[0252] Quantitation of the activity of proteins requiring Fe-S clusters can be done in an assay format. If the protein is an enzyme, such as DHAD, the activity is typically expressed in terms of units of activity. A unit of enzyme activity has been defined by the Enzyme Commission of the International Union of Biochemistry as the amount of enzyme that will catalyze the transformation of 1 micromole of the substrate per minute under standard conditions (International Union of Biochemistry, Report of the Commission on Enzymes, Oxford: Pergamon Press, 1961). Further, the term specific activity is defined as the units of activity in a given amount of enzyme. Thus, the specific activity is not directly measured but is calculated by dividing 1) the activity in units/ml of the enzyme sample by 2) the concentration of protein in that sample, so the specific activity is expressed as units/mg. The specific activity of a sample of pure, fully active enzyme is a characteristic of that enzyme. The specific activity of a sample of a mixture of proteins is a measure of the relative fraction of protein in that sample that is composed of the active enzyme of interest. DHAD activity can be measured spectrophotometrically in an end point assay using the 2,4-dinitrophenylhydrazine (2,4-DNPH) method as described in Flint, D.H. and M.H. Emptage, *J. Biol. Chem. 263*:3558-64 (1988). In this assay, the 2,4-DNPH reacts with the keto group of the 2-ketoisovaleric acid product to form a hydrazone, which is detected by its absorbance at 550 nm. The assay buffer contains 50 mM Tris-HC1, 10 mM $MgCl_2$, pH 8.0 (TM8 buffer). Sufficient 2,3-dihydroxyisovaleric acid is added to the assay buffer so that its final concentration in the assay mix is 10 mM. In each assay, an enzyme containing solution and sufficient substrate containing buffer are mixed so that the final volume is 1 ml. The assay mixture is normally incubated at 37°C for 30 minutes.

[0253] The assay is stopped by adding 250 μl of 10% (W/V) trichloroacetic acid. A few minutes later, 500 μl of a saturated solution of 2,4-DNPH in 1 N HCl is added. The mixture is incubated at room temperature for at least 10 min to allow formation of the hydrazone. Next, 1.75 ml of NaOH is added to solubilize the hydrazone and to precipitate unreacted 2,4-DNPH. A few minutes after the NaOH is added, the assay tubes are placed in a sonicator bath for 10 min to degas. The tubes are then centrifuged in a desk top centrifuge at top speed for 2 min to sediment the precipitate.

[0254] The absorbance of the supernatant is then read at 550 nm within 1 hour. The absorbance of the sample assays minus the control assays are divided by 2600 (determined from an α-ketoisovaleric acid standard curve) to find the units of enzyme activity in the assay. This assay was used in the Examples described herein in which DHAD specific activity was determined.

[0255] **Example 8. Purification and Characterization of DHAD from *S. mutans* expressed in *E. coli*.**

[0256] DHAD from *S. mutans* was purified and characterized as follows. Six liters of culture of the *E. coli* Turner strain harboring the pET28a plasmid containing the *S. mutans ilvD* gene were grown and induced with IPTG. The *S. mutans* DHAD was purified by breaking the cells with a sonicator in TM8 buffer (*see* Example 7), centrifuging the crude extract to remove cell debris, then loading the supernatant of the crude extract on a Q Sepharose (GE Healthcare) column and eluting the DHAD with an increasing concentration of NaCl in TM8 buffer. The fractions containing DHAD were pooled, brought to 1 M $(NH_4)_2SO_4$, and loaded onto a Phenyl-Sepharose column (GE Healthcare) equilibrated with 1 M $(NH_4)_2SO_4$. The DHAD was eluted with a decreasing concentration of $(NH_4)_2SO_4$. The fractions containing DHAD were pooled, concentrated to ≤ 10 ml, loaded onto a 35 x 600 cm Superdex-200 column (577 ml bed volume) (GE Healthcare) column, and eluted with TM8 buffer. As judged by SDS gels, the purity of the *S. mutans* DHAD eluted from the Superdex column was estimated to be ≥ 90%.

[0257] The UV-visible spectrum of the purified *S. mutans* DHAD is shown in Figure 3. The number of peaks above 300 nm is typical of proteins with [2Fe-2S] clusters. The *S. mutans* DHAD was reduced with sodium dithionite, and its EPR spectra was measured at

various temperatures. Figure 4 shows the EPR spectra measured at temperatures between 20°K and 70°K. The EPR spectrum of the *S. mutans* DHAD is measureable up to 70°K, which indicates that it contains a [2Fe-2S] cluster and not a [4Fe-4S] cluster because the EPR spectra of proteins containing [4Fe-4S] clusters are not observable at temperatures much above 10°K.

[0258] The exact protein content of the batch of purified *S. mutans* DHAD with the highest specific activity using the Bradford protein assay was determined by quantitative amino acid analysis. Combining the activity with the protein content gave a specific activity of 100 units/mg for this batch. The iron content of this batch determined by ICP-MS using methodology known in the art was 2 molecules of iron per molecule of DHAD. This is consistent with this batch of *S. mutans* DHAD containing a full complement of [2Fe-2S] clusters.

[0259] **Example 9. Separating the forms of DHAD in yeast crude extract from other proteins in the cell and from each other to measure the amount of DHAD present.**

[0260] DHAD protein in yeast cells exists in the forms of dimers with two Fe-S clusters/dimer, one Fe-S cluster/dimer, and zero Fe-S clusters/dimer. A method to measure the concentration of these three forms of DHAD protein in yeast crude extracts was developed using a Mono Q column and a Source 15 PHE PE 4.6/100 column (both columns obtained from GE Healthcare), and is described below.

[0261] Frozen yeast cells were thawed, suspended in 50 mM Tris-HC1, 10 mM $MgCl_2$, pH 8.0 (TM8), then broken by bead beating. The broken cells are centrifuged to remove the cell debris and generate the yeast crude extract.

[0262] The crude extract was loaded onto a 4 ml Mono Q column attached to an AKTA chromatographic system (GE Healthcare) with the A buffer being TM8 and B buffer being TM8 containing 0.5 M NaCl. The column was equilibrated with A buffer before the sample was loaded. The *S. mutans* DHAD bound to the Mono Q column under these conditions. After the sample was loaded onto the column, the column was washed with 10 mL of TM8 buffer, then the concentration of NaCl in the eluant was increased to 0.22 M NaCl. This was followed by a 30 mL linear gradient from 0.22 M to 0.35 M NaCl. During chromatography, the A_{215} of the column eluate was monitored, and 1 mL fractions

were collected. The fractions were assayed for DHAD activity. The sum of the activity of the DHAD in the fractions off the Mono Q column was close to that in the crude extract. Good separations using this column were obtained with as much as 5 mL of crude extract representing up to 1 g of yeast cell paste. The DHAD containing fractions were pooled and made 1.35 M in $(NH_4)_2SO_4$ in preparation for chromatography on the PHE column.

[0263] The Source 15 PHE PE 4.6/100 column was also attached to an AKTA chromatographic system with the A buffer being TM8 containing 1.5 M $(NH_4)_2SO_4$ and the B buffer being TM8. Before each run the column was equilibrated with 90% A. The pooled fractions from the Mono Q column made 1.35 M in $(NH_4)_2SO_4$ were loaded onto the PHE column, and at this $(NH_4)_2SO_4$ concentration, the DHAD bound to the column. During chromatography, the A_{215} of the column eluate was monitored, and 1 mL fractions were collected. The DHAD eluted from the column in three peaks when the column was developed with a 30 mL decreasing linear gradient of $(NH_4)_2SO_4$ from 1.35 M to 0 M. The area of each of the DHAD peaks was determined by integration. This elution scheme was found to be ideal for separating *S. mutans* DHAD from other yeast proteins that co-eluted with it off the Mono Q column. SDS gels run on fractions where the peaks eluted showed that well over 90% of the protein present in these peaks was DHAD when it was expressed at 1% of the soluble protein in yeast cells. The fractions containing each of the three DHAD peaks were pooled separately. Based on the UV-visible absorbent spectrum and the iron and sulfide contents of the DHAD in these peaks, it was determined that the first peak contained DHAD with two [2Fe-2S] clusters/dimers, the second peak contained DHAD with one [2Fe-2S] cluster/dimer, and the third peak contained DHAD with zero [2Fe-2S] clusters/dimers. Thus, in its native state, the *S. mutans* DHAD enzyme appears to exist as a dimer of two monomeric DHAD proteins.

[0264] A standard curve relating the amount of DHAD present in a sample to the sum of the area of the three DHAD peaks off the PHE column was obtained as follows. Crude extract from yeast cells containing no *S. mutans* DHAD was spiked with various amounts of purified *S. mutans* DHAD. These extracts were subjected to chromatography on the Mono Q and PHE columns as described above. The area under each of the three DHAD peaks was integrated. The sum of these areas was plotted against the amount of pure DHAD spiked into the yeast crude extracts. The plot was used to derive the following equation:

[0265] #μg DHAD in sample of crude extract = 0.507 x (summed area counts of the three DHAD peaks)

[0266] The DHAD activity in a crude extract of yeast can be readily determined by the method described in Example 7. The amount of DHAD protein in yeast crude extracts can be determined by the procedure outlined in this Example. With this data, one can calculate the specific activity of the *S. mutans* DHAD protein *per se* in crude extracts according to the procedure in Example 10.

[0267] **Example 10. Methods to determine the fraction of DHAD in yeast crude extract loaded with Fe-S clusters.**

[0268] When a purified Fe-S cluster requiring protein contains a full complement of clusters, it will have a characteristic specific activity. As previously mentioned, for *S. mutans* DHAD this specific activity is 100 units/mg when it has a full complement of clusters.

[0269] A DHAD sample that has on average one Fe-S cluster/per dimer could contain some dimers with two clusters, some dimers with one cluster, and some dimers with no clusters. Alternatively, if cluster addition to a dimer is all or none and on average there is one Fe-S cluster/dimer in a sample, half of the DHAD dimers would have a full complement of clusters and half would be without clusters. From the results in Example 9, we know that all or none behavior is not followed by *S. mutans* DHAD because a species with one cluster per dimer can be isolated. We have found that dimers of *S. mutans* DHAD that have one Fe-S cluster have ½ the activity of dimers with two Fe-S clusters/dimer, *i.e.*, the specific activity of *S. mutans* DHAD with ½ of a full complement of Fe-S clusters is 50 units/mg. This means the absence of an Fe-S cluster in one of the monomers of a dimer does not influence the activity of the other monomer should it contain an Fe-S cluster.

[0270] With the information obtained with the procedures described in Example 9 and the information described so far in this Example, one can determine the degree of Fe-S cluster loading in a DHAD sample in two different ways.

[0271] First, one can compare the ratio of the amounts of the three DHAD peaks to determine the relative amount that has two clusters per dimer, one cluster per dimer, and zero clusters per dimer. This gives the degree of cluster loading. For example, if the area of peak 1 off the PHE column was 25%, peak 2 was 50%, and peak 3 was 25% of the sum of the areas of peak 1, peak 2, and peak 3, the percent of the monomers loaded with clusters can be calculated to be 50% according to the following equation:

[0272] 100* [2 * (area of peak 1) + 1 * (area of peak 2) + 0 * (area of peak 3)]/[2 * (total peak area)] = % DHAD monomers with Fe-S clusters.

[0273] Second, one can use the specific activity of the DHAD present to calculate the degree of cluster loading. One determines the specific activity by dividing the activity determined as described in Example 7 with the amount of DHAD protein determined as described in Example 9. The specific activity is then divided by 100 U/mg to determine the fraction of monomers loaded with clusters. This fraction is multiplied by 100 to determine the percent DHAD monomers with Fe-S clusters.

[0274] For example if the specific activity is found to be 50 U/mg, the fraction loaded with clusters is 0.5 and the percent DHAD monomers with Fe-S clusters is 50%.

[0275] To make such a calculation, the specific activity must be based on the concentration of the DHAD protein in the crude extract (not the total protein). Determining the concentration of *S. mutans* DHAD in the presence of other proteins can be accomplished using methods described in Example 9.

[0276] **Example 11. Specific activities and inferred fraction of the DHAD-loaded proteins.**

[0277] To determine the fraction of DHAD monomers loaded with Fe-S clusters in several yeast strains grown under different conditions, the methods described above were used. Results are shown in Table 15.

Table 15. Specific Activities and Inferred Fraction of the DHAD Loaded Proteins.

BY Yeast Strain	Growth Conditions	DHAD SA in Crude Extracts (U/mg)	%DHAD is of Crude Extract Protein	% Cluster Occupancy of DHAD
WT	- Ura	0.46	2.3	10
ΔFRA2	- Ura	0.8	2.5	14

ΔGRX3	- Ura	0.99	2.4	23
WT	- Leu	0.82	11	7
ΔFRA2	- Leu	2.2	11	19
ΔGRX3	- Leu	3.5	9.5	31

[0278] These results indicate that under these growth conditions, the level of Fe-S cluster loading in the DHAD in strains lacking FRA2 and GRX3 is higher than in strains containing functional copies of these genes. Thus, a higher fraction of the DHAD protein is in the active form in the deletion strains because the content of Fe-S clusters (which are required for activity) is higher.

[0279] **Example 12. Construction of *Saccharomyces cerevisiae* strains PNY1505, PNY1541, and PNY1542.**

[0280] The purpose of this Example was to construct *Saccharomyces cerevisiae* strains PNY1505, PNY1541, and PNY1542. These strains were derived from PNY1503 (BP1064). PNY1503 was derived from CEN.PK 113-7D (CBS 8340; Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversiry Centre, Netherlands). The construction of PNY1503 (BP1064) is described in U.S. Appl. No. 61/368,436, incorporated by reference herein, and in Example 13 below. PNY1505 contains a deletion of the *FRA2* gene. PNY1541 and PNY1542 contain an integration of the *AFT1* gene with the L99A mutation (*AFT1-L99A*) at the *YPRCΔ15* locus.

[0281] Deletions/integrations were created by homologous recombination with PCR fragments containing regions of homology upstream and downstream of the target gene and the *URA3* gene for selection of transformants. The *URA3* gene was removed by homologous recombination to create a scarless deletion/integration.

[0282] The scarless deletion/integration procedure was adapted from Akada *et al*., *Yeast, 23*(5):399-405 (2006). The PCR cassette for each deletion/integration was made by combining four fragments, A-B-U-C, either by overlapping PCR or by cloning the individual fragments, and gene to be integrated, into a plasmid prior to amplifying the entire cassette by PCR for the deletion/integration procedure. The PCR cassette contained a selectable/counter-selectable marker, *URA3* (Fragment U), consisting of the native CEN.PK 113-7D *URA3* gene, along with the promoter (250 bp upstream of the *URA3* gene) and terminator (150 bp downstream of the *URA3* gene) regions. Fragments

A (150 bp to 500 bp long) and C (250 bp long) corresponded to the sequence immediately upstream of the target gene (Fragment A) and the 3' sequence of the target gene (Fragment C). Fragments A and C were used for integration of the cassette into the chromosome by homologous recombination. Fragment B (500 bp long) corresponded to the 500 bp immediately downstream of the target gene and was used for excision of the *URA3* marker and Fragment C from the chromosome by homologous recombination, as a direct repeat of the sequence corresponding to Fragment B was created upon integration of the cassette into the chromosome.

[0283] Using the PCR product ABUC cassette, the *URA3* marker was first integrated into and then excised from the chromosome by homologous recombination. The initial integration deleted the gene, excluding the 3' sequence. Upon excision, the 3' region of the gene was also deleted. For integration of genes using this method, the gene to be integrated was included in the cassette between fragments A and B.

[0284] *FRA2* Deletion

[0285] The *FRA2* deletion (also described in U.S. Appl. No. 61/380,563, incorporated by reference herein) was designed to delete 250 nucleotides from the 3' end of the coding sequence, leaving the first 113 nucleotides of the FRA2 coding sequence intact. An in-frame stop codon was present 7 nucleotides downstream of the deletion. The four fragments for the PCR cassette for the scarless FRA2 deletion were amplified using Phusion High Fidelity PCR Master Mix (New England BioLabs; Ipswich, MA) and CEN.PK 113-7D genomic DNA as template, prepared with a Gentra Puregene Yeast/Bact kit (Qiagen; Valencia, CA). FRA2 Fragment A was amplified with primer oBP594 (SEQ ID NO: 961) and primer oBP595 (SEQ ID NO: 962), containing a 5' tail with homology to the 5' end of FRA2 Fragment B. FRA2 Fragment B was amplified with primer oBP596 (SEQ ID NO: 963), containing a 5' tail with homology to the 3' end of FRA2 Fragment A, and primer oBP597 (SEQ ID NO: 964), containing a 5' tail with homology to the 5' end of FRA2 Fragment U. FRA2 Fragment U was amplified with primer oBP598 (SEQ ID NO: 965), containing a 5' tail with homology to the 3' end of FRA2 Fragment B, and primer oBP599 (SEQ ID NO: 966), containing a 5' tail with homology to the 5' end of FRA2 Fragment C. FRA2 Fragment C was amplified with primer oBP600 (SEQ ID NO: 967), containing a 5' tail with homology to the 3' end of FRA2 Fragment U, and primer oBP601 (SEQ ID NO: 968). PCR products were purified with a

PCR Purification kit (Qiagen). FRA2 Fragment AB was created by overlapping PCR by mixing FRA2 Fragment A and FRA2 Fragment B and amplifying with primers oBP594 (SEQ ID NO: 961) and oBP597 (SEQ ID NO: 964). FRA2 Fragment UC was created by overlapping PCR by mixing FRA2 Fragment U and FRA2 Fragment C and amplifying with primers oBP598 (SEQ ID NO: 965) and oBP601 (SEQ ID NO: 968). The resulting PCR products were purified on an agarose gel followed by a Gel Extraction kit (Qiagen). The FRA2 ABUC cassette was created by overlapping PCR by mixing FRA2 Fragment AB and FRA2 Fragment UC and amplifying with primers oBP594 (SEQ ID NO: 961) and oBP601 (SEQ ID NO: 968). The PCR product was purified with a PCR Purification kit (Qiagen).

[0286] Competent cells of PNY1503 were made and transformed with the FRA2 ABUC PCR cassette using a Frozen-EZ Yeast Transformation II kit (Zymo Research; Orange, CA). Transformation mixtures were plated on synthetic complete media lacking uracil supplemented with 1% ethanol at 30°C. Transformants with a *fra2* knockout were screened for by PCR with primers oBP602 (SEQ ID NO: 969) and oBP603 (SEQ ID NO: 970) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). A correct transformant was grown in YPE (yeast extract, peptone, 1% ethanol) and plated on synthetic complete medium supplemented with 1% ethanol and containing 5-fluoro-orotic acid (0.1%) at 30°C to select for isolates that lost the URA3 marker. The deletion and marker removal were confirmed by PCR with primers oBP602 (SEQ ID NO: 969) and oBP603 (SEQ ID NO: 970) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). The absence of the *FRA2* gene from the isolate was demonstrated by a negative PCR result using primers specific for the deleted coding sequence of *FRA2*, oBP605 (SEQ ID NO: 971) and oBP606 (SEQ ID NO: 972). The correct isolate was selected as strain CEN.PK 113-7D MATa ura3Δ::loxP his3Δ pdc6Δ pdc1Δ::P[PDC1]-DHAD|ilvD_Sm-PDC1t pdc5Δ::P[PDC5]-ADH|sadB_Ax-PDC5t gpd2Δ::loxP fra2Δ and designated as PNY1505 (BP1135).

Table 16. Primers used in the *FRA2* Deletion

Primer Name	SEQ ID NO	Primer Sequence
oBP594	961	agctgtctcgtgttgtgggttt
oBP595	962	cttaataatagaacaatatcatcctttacgggcatcttatagtgtcgtt
oBP596	963	gcgccaacgacactataagatgcccgtaaaggatgatattgttctatta
oBP597	964	tatggaccctgaaaccacagccacattgcaacgacgacaatgccaaacc

oBP598	965	tccttggtttggcattgtcgtcgttgcaatgtggctgtggtttcagggt
oBP599	966	atcctctcgcggagtccctgttcagtaaaggccatgaagctttttcttt
oBP600	967	attggaaagaaaaagcttcatggcctttactgaacagggactccgcgag
oBP601	968	tcataccacaatcttagaccat
oBP602	969	tgttcaaacccctaaccaacc
oBP603	970	tgttcccacaatctattaccta
oBP605	971	tactgaacagggactccgcga
oBP606	972	tcataccacaatcttagacca

[0287] *YPRCΔ15* Deletion and *AFT1-L99A* Integration

[0288] The YPRCΔ15 locus was deleted and replaced with AFT1-L99A along with the native promoter region (800 bp) and terminator region (800 bp) from AFT1. The scarless cassette for the YPRCΔ15 deletion- AFT1L99A integration was first cloned into plasmid pUC19-URA3MCS (described in U.S. Appl. No. 61/356,379, incorporated by reference herein). The vector is pUC19 based and contains the sequence of the URA3 gene from *S. cerevisiae* CEN.PK 113-7D situated within a multiple cloning site (MCS). pUC19 (American Type Culture Collection, Manassas, VA; ATCC# 37254) contains the pMB1 replicon and a gene coding for beta-lactamase for replication and selection in *Escherichia coli*. In addition to the coding sequence for URA3, the sequences from upstream (250 bp) and downstream (150 bp) of this gene are present for expression of the URA3 gene in yeast. The vector can be used for cloning purposes and can be used as a yeast integration vector.

[0289] The DNA encompassing the URA3 coding region along with 250 bp upstream and 150 bp downstream of the URA3 coding region from *Saccaromyces cerevisiae* CEN.PK 113-7D (CBS 8340; Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, Netherlands) genomic DNA was amplified with primers oBP438 (SEQ ID NO: 1033), containing *BamH*I, *Asc*I, *Pme*I, and *Fse*I restriction sites, and oBP439 (SEQ ID NO: 1034), containing *Xba*I, *Pac*I, and *Not*I restriction sites. Genomic DNA was prepared using a Gentra Puregene Yeast/Bact kit (Qiagen). The PCR product and pUC19 were ligated with T4 DNA ligase after digestion with *BamH*I and *Xba*I to create vector pUC19-URA3MCS. The vector was confirmed by PCR and sequencing with primers oBP264 (SEQ ID NO:1031) and oBP265 (SEQ ID NO: 1032).

[0290] YPRCΔ15 Fragment A was amplified from genomic DNA, prepared as above, with primer oBP622 (SEQ ID NO: 973), containing a KpnI restriction site, and primer oBP623 (SEQ ID NO: 974), containing a 5' tail with homology to the 5' end of

YPRCΔ15 Fragment B. YPRCΔ15 Fragment B was amplified from genomic DNA with primer oBP624 (SEQ ID NO: 975), containing a 5' tail with homology to the 3' end of YPRCΔ15 Fragment A, and primer oBP625 (SEQ ID NO: 976), containing a FseI restriction site. PCR products were purified with a PCR Purification kit (Qiagen). YPRCΔ15 Fragment A - YPRCΔ15 Fragment B was created by overlapping PCR by mixing the YPRCΔ15 Fragment A and YPRCΔ15 Fragment B PCR products and amplifying with primers oBP622 (SEQ ID NO: 973) and oBP625 (SEQ ID NO: 976). The resulting PCR product was digested with KpnI and FseI and ligated with T4 DNA ligase into the corresponding sites of pUC19-URA3MCS after digestion with the appropriate enzymes. YPRCΔ15 Fragment C was amplified from genomic DNA with primer oBP626 (SEQ ID NO: 977), containing a NotI restriction site, and primer oBP627 (SEQ ID NO: 978), containing a PacI restriction site. The YPRCΔ15 Fragment C PCR product was digested with NotI and PacI and ligated with T4 DNA ligase into the corresponding sites of the plasmid containing YPRCΔ15 Fragments AB. *AFT1-L99A*, along with the native promoter region (800 bp) and terminator region (800 bp) from *AFT1*, was amplified using pRS411-AFT1(L99A) (described in Example 4 above) as template with primer oBP744 (SEQ ID NO: 979), containing an AscI restriction site, and primer oBP745 (SEQ ID NO: 980), containing a PmeI restriction site. The PCR product was digested with AscI and PmeI and ligated with T4 DNA ligase into the corresponding sites of the plasmid containing YPRCΔ15 Fragments ABC. The entire integration cassette was amplified from the resulting plasmid with primers oBP622 (SEQ ID NO: 973) and oBP627 (SEQ ID NO: 978).

[0291] Competent cells of PNY1503 were made and transformed with the *YPRCΔ15* deletion/*AFT1-L99A* integration cassette PCR product using a Frozen-EZ Yeast Transformation II kit (Zymo Research). Transformation mixtures were plated on synthetic complete media lacking uracil supplemented with 1% ethanol at 30°C. Transformants were grown in YPE (1% ethanol) and plated on synthetic complete medium supplemented with 1% EtOH and containing 5-fluoro-orotic acid (0.1%) at 30°C to select for isolates that lost the *URA3* marker. The deletion of *YPRCΔ15* and integration of *AFT1L99A* were confirmed by PCR with external primers oBP636 (SEQ ID NO: 981) and oBP637 (SEQ ID NO: 982) and with *AFT1-L99A* specific primer HY840 (SEQ ID NO: 983) and external primer oBP637 (SEQ ID NO: 982) using genomic DNA prepared

with a Gentra Puregene Yeast/Bact kit (Qiagen) and by colony PCR. Correct independent isolates of CEN.PK 113-7D MATa ura3Δ::loxP his3Δ pdc6Δ pdc1Δ::P[PDC1]-DHAD|ilvD_Sm-PDC1t pdc5Δ::P[PDC5]-ADH|sadB_Ax-PDC5t gpd2Δ::loxP yprcΔ15Δ::AFT1L99A were designated as strains PNY1541 and PNY1542.

Table 17. Primers used in the *YPRCΔ15* Deletion and *AFT1-L99A* Integration

Primer Name	SEQ ID NO	Primer Sequence
oBP622	973	aattggtaccccaaaaggaatattgggtcaga
oBP623	974	ccattgtttaaacggcgcgccggatcctttgcgaaaccctatgctctgt
oBP624	975	gcaaaggatccggcgcgccgtttaaacaatggaaggtcgggatgagcat
oBP625	976	aattggccggcctacgtaacattctgtcaaccaa
oBP626	977	aattgcggccgcttcatatatgacgtaataaaat
oBP627	978	aattttaattaatttttttcttggaatcagtac
oBP744	979	aattggcgcgccagagtacaacgatcaccgcctg
oBP745	980	aattgtttaaacgaacgaaagttacaaaatctag
oBP636	981	catttttttccctctaagaagc
oBP637	982	tttttgcacagttaaactaccc
HY840	983	CCAAAATCAGCCCCACGACGGCCATA

[0292] Example 13. Construction of *Saccharomyces cerevisiae* strain BP1064 (PNY1503).

[0293] The strain BP1064 was derived from CEN.PK 113-7D (CBS 8340; Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, Netherlands) and contains deletions of the following genes: URA3, HIS3, PDC1, PDC5, PDC6, and GPD2.

[0294] Deletions, which completely removed the entire coding sequence, were created by homologous recombination with PCR fragments containing regions of homology upstream and downstream of the target gene and either a G418 resistance marker or URA3 gene for selection of transformants. The G418 resistance marker, flanked by loxP sites, was removed using Cre recombinase. The URA3 gene was removed by homologous recombination to create a scarless deletion, or if flanked by loxP sites was removed using Cre recombinase.

[0295] The scarless deletion procedure was adapted from Akada *et al.* 2006 *Yeast* v23 p399. In general, the PCR cassette for each scarless deletion was made by combining four fragments, A-B-U-C, by overlapping PCR. The PCR cassette contained a

selectable/counter-selectable marker, URA3 (Fragment U), consisting of the native CEN.PK 113-7D URA3 gene, along with the promoter (250 bp upstream of the URA3 gene) and terminator (150 bp downstream of the URA3 gene). Fragments A and C, each 500 bp long, corresponded to the 500 bp immediately upstream of the target gene (Fragment A) and the 3' 500 bp of the target gene (Fragment C). Fragments A and C were used for integration of the cassette into the chromosome by homologous recombination. Fragment B (500 bp long) corresponded to the 500 bp immediately downstream of the target gene and was used for excision of the URA3 marker and Fragment C from the chromosome by homologous recombination, as a direct repeat of the sequence corresponding to Fragment B was created upon integration of the cassette into the chromosome. Using the PCR product ABUC cassette, the URA3 marker was first integrated into and then excised from the chromosome by homologous recombination. The initial integration deleted the gene, excluding the 3' 500 bp. Upon excision, the 3' 500 bp region of the gene was also deleted. For integration of genes using this method, the gene to be integrated was included in the PCR cassette between fragments A and B.

[0296] URA3 Deletion

[0297] To delete the endogenous URA3 coding region, a *ura3::loxP-kanMX-loxP* cassette was PCR-amplified from pLA54 template DNA (SEQ ID NO: 986). pLA54 contains the *K. lactis* TEF1 promoter and kanMX marker, and is flanked by loxP sites to allow recombination with Cre recombinase and removal of the marker. PCR was done using Phusion DNA polymerase and primers BK505 and BK506 (SEQ ID NOs: 987 and 988, respectively). The *URA3* portion of each primer was derived from the 5' region upstream of the *URA3* promoter and 3' region downstream of the coding region such that integration of the *loxP-kanMX-loxP* marker resulted in replacement of the *URA3* coding region. The PCR product was transformed into CEN.PK 113-7D using standard genetic techniques (*Methods in Yeast Genetics*, 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 201-202) and transformants were selected on YPD containing G418 (100 μg/ml) at 30 C. Transformants were screened to verify correct integration by PCR using primers LA468 and LA492 (SEQ ID NOs: 989 and 990, respectively) and designated CEN.PK 113-7D Δura3::kanMX.

[0298] HIS3 Deletion

[0299] The four fragments for the PCR cassette for the scarless HIS3 deletion were amplified using Phusion High Fidelity PCR Master Mix (New England BioLabs; Ipswich, MA) and CEN.PK 113-7D genomic DNA as template, prepared with a Gentra Puregene Yeast/Bact kit (Qiagen; Valencia, CA). HIS3 Fragment A was amplified with primer oBP452 (SEQ ID NO: 991) and primer oBP453 (SEQ ID NO: 992), containing a 5' tail with homology to the 5' end of HIS3 Fragment B. HIS3 Fragment B was amplified with primer oBP454 (SEQ ID NO: 993), containing a 5' tail with homology to the 3' end of HIS3 Fragment A, and primer oBP455 (SEQ ID NO: 994), containing a 5' tail with homology to the 5' end of HIS3 Fragment U. HIS3 Fragment U was amplified with primer oBP456 (SEQ ID NO: 995), containing a 5' tail with homology to the 3' end of HIS3 Fragment B, and primer oBP457 (SEQ ID NO: 996), containing a 5' tail with homology to the 5' end of HIS3 Fragment C. HIS3 Fragment C was amplified with primer oBP458 (SEQ ID NO: 997), containing a 5' tail with homology to the 3' end of HIS3 Fragment U, and primer oBP459 (SEQ ID NO: 998). PCR products were purified with a PCR Purification kit (Qiagen). HIS3 Fragment AB was created by overlapping PCR by mixing HIS3 Fragment A and HIS3 Fragment B and amplifying with primers oBP452 (SEQ ID NO: 991) and oBP455 (SEQ ID NO: 994). HIS3 Fragment UC was created by overlapping PCR by mixing HIS3 Fragment U and HIS3 Fragment C and amplifying with primers oBP456 (SEQ ID NO: 995) and oBP459 (SEQ ID NO: 998). The resulting PCR products were purified on an agarose gel followed by a Gel Extraction kit (Qiagen). The HIS3 ABUC cassette was created by overlapping PCR by mixing HIS3 Fragment AB and HIS3 Fragment UC and amplifying with primers oBP452 (SEQ ID NO: 991) and oBP459 (SEQ ID NO: 998). The PCR product was purified with a PCR Purification kit (Qiagen).

[0300] Competent cells of CEN.PK 113-7D Δura3::kanMX were made and transformed with the HIS3 ABUC PCR cassette using a Frozen-EZ Yeast Transformation II kit (Zymo Research; Orange, CA). Transformation mixtures were plated on synthetic complete media lacking uracil supplemented with 2% glucose at 30°C. Transformants with a his3 knockout were screened for by PCR with primers oBP460 (SEQ ID NO: 999) and oBP461 (SEQ ID NO: 1000) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). A correct transformant was selected as strain CEN.PK 113-7D Δura3::kanMX Δhis3::URA3.

[0301] KanMX Marker Removal from the Δura3 Site and URA3 Marker Removal from the Δhis3 Site

[0302] The KanMX marker was removed by transforming CEN.PK 113-7D Δura3::kanMX Δhis3::URA3 with pRS423::PGAL1-cre (SEQ ID NO: 1011, described in US Provisional Application No. 61/290,639) using a Frozen-EZ Yeast Transformation II kit (Zymo Research) and plating on synthetic complete medium lacking histidine and uracil supplemented with 2% glucose at 30°C. Transformants were grown in YP supplemented with 1% galactose at 30°C for ~6 hours to induce the Cre recombinase and KanMX marker excision and plated onto YPD (2% glucose) plates at 30°C for recovery. An isolate was grown overnight in YPD and plated on synthetic complete medium containing 5-fluoro-orotic acid (0.1%) at 30°C to select for isolates that lost the URA3 marker. 5-FOA resistant isolates were grown in and plated on YPD for removal of the pRS423::P_{GAL1}-*cre* plasmid. Isolates were checked for loss of the KanMX marker, URA3 marker, and pRS423::P_{GAL1}-*cre* plasmid by assaying growth on YPD+G418 plates, synthetic complete medium lacking uracil plates, and synthetic complete medium lacking histidine plates. A correct isolate that was sensitive to G418 and auxotrophic for uracil and histidine was selected as strain CEN.PK 113-7D Δura3::loxP Δhis3 and designated as BP857. The deletions and marker removal were confirmed by PCR and sequencing with primers oBP450 (SEQ ID NO: 1001) and oBP451 (SEQ ID NO: 1002) for Δura3 and primers oBP460 (SEQ ID NO: 999) and oBP461 (SEQ ID NO: 1000) for Δhis3 using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen).

[0303] PDC6 Deletion

[0304] The four fragments for the PCR cassette for the scarless PDC6 deletion were amplified using Phusion High Fidelity PCR Master Mix (New England BioLabs) and CEN.PK 113-7D genomic DNA as template, prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). PDC6 Fragment A was amplified with primer oBP440 (SEQ ID NO: 1003) and primer oBP441 (SEQ ID NO: 1004), containing a 5' tail with homology to the 5' end of PDC6 Fragment B. PDC6 Fragment B was amplified with primer oBP442 (SEQ ID NO: 1005), containing a 5' tail with homology to the 3' end of PDC6 Fragment A, and primer oBP443 (SEQ ID NO: 1006), containing a 5' tail with homology to the 5' end of PDC6 Fragment U. PDC6 Fragment U was amplified with primer oBP444 (SEQ ID NO: 1007), containing a 5' tail with homology to the 3' end of PDC6 Fragment B, and primer

oBP445 (SEQ ID NO: 1008), containing a 5' tail with homology to the 5' end of PDC6 Fragment C. PDC6 Fragment C was amplified with primer oBP446 (SEQ ID NO: 1009), containing a 5' tail with homology to the 3' end of PDC6 Fragment U, and primer oBP447 (SEQ ID NO: 1010). PCR products were purified with a PCR Purification kit (Qiagen). PDC6 Fragment AB was created by overlapping PCR by mixing PDC6 Fragment A and PDC6 Fragment B and amplifying with primers oBP440 (SEQ ID NO: 1003) and oBP443 (SEQ ID NO: 1006). PDC6 Fragment UC was created by overlapping PCR by mixing PDC6 Fragment U and PDC6 Fragment C and amplifying with primers oBP444 (SEQ ID NO: 1007) and oBP447 (SEQ ID NO: 1010). The resulting PCR products were purified on an agarose gel followed by a Gel Extraction kit (Qiagen). The PDC6 ABUC cassette was created by overlapping PCR by mixing PDC6 Fragment AB and PDC6 Fragment UC and amplifying with primers oBP440 (SEQ ID NO: 1003) and oBP447 (SEQ ID NO: 1010). The PCR product was purified with a PCR Purification kit (Qiagen).

[0305] Competent cells of CEN.PK 113-7D Δura3::loxP Δhis3 were made and transformed with the PDC6 ABUC PCR cassette using a Frozen-EZ Yeast Transformation II kit (Zymo Research). Transformation mixtures were plated on synthetic complete media lacking uracil supplemented with 2% glucose at 30°C. Transformants with a pdc6 knockout were screened for by PCR with primers oBP448 (SEQ ID NO: 1012) and oBP449 (SEQ ID NO: 1013) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). A correct transformant was selected as strain CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6::URA3.

[0306] CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6::URA3 was grown overnight in YPD and plated on synthetic complete medium containing 5-fluoro-orotic acid (0.1%) at 30°C to select for isolates that lost the URA3 marker. The deletion and marker removal were confirmed by PCR and sequencing with primers oBP448 (SEQ ID NO: 1012) and oBP449 (SEQ ID NO: 1013) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). The absence of the PDC6 gene from the isolate was demonstrated by a negative PCR result using primers specific for the coding sequence of PDC6, oBP554 (SEQ ID NO: 1014) and oBP555 (SEQ ID NO: 1015). The correct isolate was selected as strain CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 and designated as BP891.

[0307] PDC1 Deletion ilvDSm Integration

[0308] The PDC1 gene was deleted and replaced with the *ilvD* coding region from *Streptococcus mutans* ATCC #700610. The A fragment followed by the *ilvD* coding region from *Streptococcus mutans* for the PCR cassette for the PDC1 deletion-ilvDSm integration was amplified using Phusion High Fidelity PCR Master Mix (New England BioLabs) and NYLA83 (described in US Provisional Application No. 61/246709) genomic DNA as template, prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). PDC1 Fragment A-ilvDSm (SEQ ID NO: 1053) was amplified with primer oBP513 (SEQ ID NO: 1016) and primer oBP515 (SEQ ID NO: 1017), containing a 5' tail with homology to the 5' end of PDC1 Fragment B. The B, U, and C fragments for the PCR cassette for the PDC1 deletion-ilvDSm integration were amplified using Phusion High Fidelity PCR Master Mix (New England BioLabs) and CEN.PK 113-7D genomic DNA as template, prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). PDC1 Fragment B was amplified with primer oBP516 (SEQ ID NO: 1018) containing a 5' tail with homology to the 3' end of PDC1 Fragment A-ilvDSm, and primer oBP517 (SEQ ID NO: 1019), containing a 5' tail with homology to the 5' end of PDC1 Fragment U. PDC1 Fragment U was amplified with primer oBP518 (SEQ ID NO: 1020), containing a 5' tail with homology to the 3' end of PDC1 Fragment B, and primer oBP519 (SEQ ID NO: 1021), containing a 5' tail with homology to the 5' end of PDC1 Fragment C. PDC1 Fragment C was amplified with primer oBP520 (SEQ ID NO: 1022), containing a 5' tail with homology to the 3' end of PDC1 Fragment U, and primer oBP521 (SEQ ID NO: 1023). PCR products were purified with a PCR Purification kit (Qiagen). PDC1 Fragment A-ilvDSm-B was created by overlapping PCR by mixing PDC1 Fragment A-ilvDSm and PDC1 Fragment B and amplifying with primers oBP513 (SEQ ID NO: 1016) and oBP517 (SEQ ID NO: 1019). PDC1 Fragment UC was created by overlapping PCR by mixing PDC1 Fragment U and PDC1 Fragment C and amplifying with primers oBP518 (SEQ ID NO: 1020) and oBP521 (SEQ ID NO: 1023). The resulting PCR products were purified on an agarose gel followed by a Gel Extraction kit (Qiagen). The PDC1 A-ilvDSm-BUC cassette (SEQ ID NO: 1054) was created by overlapping PCR by mixing PDC1 Fragment A-ilvDSm-B and PDC1 Fragment UC and amplifying with primers oBP513 (SEQ ID NO: 1016) and oBP521 (SEQ ID NO: 1023). The PCR product was purified with a PCR Purification kit (Qiagen).

[0309] Competent cells of CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 were made and transformed with the PDC1 A-ilvDSm-BUC PCR cassette using a Frozen-EZ Yeast Transformation II kit (Zymo Research). Transformation mixtures were plated on synthetic complete media lacking uracil supplemented with 2% glucose at 30°C. Transformants with a pdc1 knockout ilvDSm integration were screened for by PCR with primers oBP511 (SEQ ID NO: 1024) and oBP512 (SEQ ID NO: 1025) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). The absence of the PDC1 gene from the isolate was demonstrated by a negative PCR result using primers specific for the coding sequence of PDC1, oBP550 (SEQ ID NO: 1026) and oBP551 (SEQ ID NO: 1027). A correct transformant was selected as strain CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 Δpdc1::ilvDSm-URA3.

[0310] CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 Δpdc1::ilvDSm-URA3 was grown overnight in YPD and plated on synthetic complete medium containing 5-fluoro-orotic acid (0.1%) at 30°C to select for isolates that lost the URA3 marker. The deletion of PDC1, integration of ilvDSm, and marker removal were confirmed by PCR and sequencing with primers oBP511 (SEQ ID NO: 1024) and oBP512 (SEQ ID NO: 1025) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). The correct isolate was selected as strain CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 Δpdc1::ilvDSm and designated as BP907.

[0311] PDC5 Deletion sadB Integration

[0312] The PDC5 gene was deleted and replaced with the *sadB* coding region from *Achromobacter xylosoxidans*. A segment of the PCR cassette for the PDC5 deletion-sadB integration was first cloned into plasmid pUC19-URA3MCS.

[0313] pUC19-URA3MCS is pUC19 based and contains the sequence of the URA3 gene from *Saccaromyces cerevisiae* situated within a multiple cloning site (MCS). pUC19 contains the pMB1 replicon and a gene coding for beta-lactamase for replication and selection in *Escherichia coli*. In addition to the coding sequence for URA3, the sequences from upstream and downstream of this gene were included for expression of the URA3 gene in yeast. The vector can be used for cloning purposes and can be used as a yeast integration vector.

[0314] The DNA encompassing the URA3 coding region along with 250 bp upstream and 150 bp downstream of the URA3 coding region from *Saccaromyces cerevisiae* CEN.PK

113-7D genomic DNA was amplified with primers oBP438 (SEQ ID NO: 1033), containing BamHI, AscI, PmeI, and FseI restriction sites, and oBP439 (SEQ ID NO: 1034), containing XbaI, PacI, and NotI restriction sites, using Phusion High-Fidelity PCR Master Mix (New England BioLabs). Genomic DNA was prepared using a Gentra Puregene Yeast/Bact kit (Qiagen). The PCR product and pUC19 (SEQ ID NO: 1056) were ligated with T4 DNA ligase after digestion with BamHI and XbaI to create vector pUC19-URA3MCS. The vector was confirmed by PCR and sequencing with primers oBP264 (SEQ ID NO: 1031) and oBP265 (SEQ ID NO: 1032).

[0315] The coding sequence of *sadB* and PDC5 Fragment B were cloned into pUC19-URA3MCS to create the sadB-BU portion of the PDC5 A-sadB-BUC PCR cassette. The coding sequence of *sadB* was amplified using pLH468-sadB (SEQ ID NO: 1051) as template with primer oBP530 (SEQ ID NO: 1035), containing an AscI restriction site, and primer oBP531 (SEQ ID NO: 1036), containing a 5' tail with homology to the 5' end of PDC5 Fragment B. PDC5 Fragment B was amplified with primer oBP532 (SEQ ID NO: 1037), containing a 5' tail with homology to the 3' end of *sadB*, and primer oBP533 (SEQ ID NO: 1038), containing a PmeI restriction site. PCR products were purified with a PCR Purification kit (Qiagen). sadB-PDC5 Fragment B was created by overlapping PCR by mixing the sadB and PDC5 Fragment B PCR products and amplifying with primers oBP530 (SEQ ID NO: 1035) and oBP533 (SEQ ID NO: 1038). The resulting PCR product was digested with AscI and PmeI and ligated with T4 DNA ligase into the corresponding sites of pUC19-URA3MCS after digestion with the appropriate enzymes. The resulting plasmid was used as a template for amplification of sadB-Fragment B-Fragment U using primers oBP536 (SEQ ID NO: 1039) and oBP546 (SEQ ID NO: 1040), containing a 5' tail with homology to the 5' end of PDC5 Fragment C. PDC5 Fragment C was amplified with primer oBP547 (SEQ ID NO: 1041) containing a 5' tail with homology to the 3' end of PDC5 sadB-Fragment B-Fragment U, and primer oBP539 (SEQ ID NO: 1042). PCR products were purified with a PCR Purification kit (Qiagen). PDC5 sadB-Fragment B-Fragment U-Fragment C was created by overlapping PCR by mixing PDC5 sadB-Fragment B-Fragment U and PDC5 Fragment C and amplifying with primers oBP536 (SEQ ID NO: 1039) and oBP539 (SEQ ID NO: 1042). The resulting PCR product was purified on an agarose gel followed by a Gel Extraction kit (Qiagen). The PDC5 A-sadB-BUC cassette (SEQ ID NO: 1055) was created by amplifying PDC5

sadB-Fragment B-Fragment U-Fragment C with primers oBP542 (SEQ ID NO: 1043), containing a 5' tail with homology to the 50 nucleotides immediately upstream of the native PDC5 coding sequence, and oBP539 (SEQ ID NO: 1042). The PCR product was purified with a PCR Purification kit (Qiagen).

[0316] Competent cells of CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 Δpdc1::ilvDSm were made and transformed with the PDC5 A-sadB-BUC PCR cassette using a Frozen-EZ Yeast Transformation II kit (Zymo Research). Transformation mixtures were plated on synthetic complete media lacking uracil supplemented with 1% ethanol (no glucose) at 30°C. Transformants with a pdc5 knockout sadB integration were screened for by PCR with primers oBP540 (SEQ ID NO: 1044) and oBP541 (SEQ ID NO: 1045) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). The absence of the PDC5 gene from the isolate was demonstrated by a negative PCR result using primers specific for the coding sequence of PDC5, oBP552 (SEQ ID NO: 1046) and oBP553 (SEQ ID NO: 1047). A correct transformant was selected as strain CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 Δpdc1::ilvDSm Δpdc5::sadB-URA3.

[0317] CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 Δpdc1::ilvDSm Δpdc5::sadB-URA3 was grown overnight in YPE (1% ethanol) and plated on synthetic complete medium supplemented with ethanol (no glucose) and containing 5-fluoro-orotic acid (0.1%) at 30°C to select for isolates that lost the URA3 marker. The deletion of PDC5, integration of *sadB*, and marker removal were confirmed by PCR with primers oBP540 (SEQ ID NO: 1044) and oBP541 (SEQ ID NO: 1045) using genomic DNA prepared with a Gentra Puregene Yeast/Bact kit (Qiagen). The correct isolate was selected as strain CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 Δpdc1::ilvDSm Δpdc5::sadB and designated as BP913.

[0318] GPD2 Deletion

[0319] To delete the endogenous GPD2 coding region, a gpd2::loxP-URA3-loxP cassette (SEQ ID NO: 1057) was PCR-amplified using loxP-URA3-loxP PCR (SEQ ID NO: 1052) as template DNA. loxP-URA3-loxP contains the URA3 marker from (ATCC # 77107) flanked by loxP recombinase sites. PCR was done using Phusion DNA polymerase and primers LA512 and LA513 (SEQ ID NOs: 1029 and 1030, respectively). The GPD2 portion of each primer was derived from the 5' region upstream of the GPD2 coding region and 3' region downstream of the coding region such that integration of the loxP-URA3-loxP marker resulted in replacement of the GPD2 coding region. The PCR

product was transformed into BP913 and transformants were selected on synthetic complete media lacking uracil supplemented with 1% ethanol (no glucose). Transformants were screened to verify correct integration by PCR using primers oBP582 and AA270 (SEQ ID NOs: 1048 and 1049, respectively).

[0320] The URA3 marker was recycled by transformation with pRS423::P_{GAL1}-*cre* (SEQ ID NO: 1011) and plating on synthetic complete media lacking histidine supplemented with 1% ethanol at 30°C. Transformants were streaked on synthetic complete medium supplemented with 1% ethanol and containing 5-fluoro-orotic acid (0.1%) and incubated at 30 C to select for isolates that lost the URA3 marker. 5-FOA resistant isolates were grown in YPE (1% ethanol) for removal of the pRS423::P_{GAL1}-*cre* plasmid. The deletion and marker removal were confirmed by PCR with primers oBP582 (SEQ ID NO: 1048) and oBP591 (SEQ ID NO: 1050). The correct isolate was selected as strain CEN.PK 113-7D Δura3::loxP Δhis3 Δpdc6 Δpdc1::ilvDSm Δpdc5::sadB Δgpd2::loxP and designated as BP1064.

[0321] **Example 14. Shake flask experiment to measure 2,3-dihydroxyisovalerate accumulation and isobutanol production.**

[0322] The purpose of this Example was to show the effect on accumulation of the isobutanol pathway intermediate 2,3-dihydroxyisovalerate (DHIV) and show isobutanol production in isobutanologen strains with an integrated copy of the *AFT1-L99A* gene or a *FRA2* deletion compared to the parent strain. Strains were transformed with isobutanol pathway plasmids pYZ090 (SEQ ID NO: 984; described in U.S. Appl. No. 61/368,436, incorporated by reference herein) and pLH468 (SEQ ID NO: 985; described in U.S. Application No. 61/246,844, incorporated by reference herein). These plasmids are also described briefly, as follows.

[0323] pYZ090 (SEQ ID NO: 984) was constructed to contain a chimeric gene having the coding region of the alsS gene from *Bacillus subtilis* (nt position 457-2172) expressed from the yeast CUP1 promoter (nt 2-449) and followed by the CYC1 terminator (nt 2181-2430) for expression of ALS, and a chimeric gene having the coding region of the ilvC gene from *Lactococcus lactis* (nt 3634-4656) expressed from the yeast ILV5 promoter

(2433-3626) and followed by the ILV5 terminator (nt 4682-5304) for expression of KARI.

[0324] pLH468 (SEQ ID NO: 985) was constructed to contain: a chimeric gene having the coding region of the ilvD gene from *Streptococcus mutans* (nt position 3313-4849) expressed from the *S. cerevisiae* FBA1 promoter (nt 2109 - 3105) followed by the FBA1 terminator (nt 4858 - 5857) for expression of DHAD; a chimeric gene having the coding region of codon optimized horse liver alcohol dehydrogenase (nt 6286-7413) expressed from the *S. cerevisiae* GPM1 promoter (nt 7425-8181) followed by the ADH1 terminator (nt 5962-6277) for expression of ADH; and a chimeric gene having the coding region of the codon-optimized kivD gene from *Lactococcus lactis* (nt 9249-10895) expressed from the TDH3 promoter (nt 10896-11918) followed by the TDH3 terminator (nt 8237-9235) for expression of KivD.

[0325] A transformant of PNY1503 (parent strain) was designated PNY1504. A transformant of PNY1505 (*fra2* deletion strain) was designated PNY1506. Transformants of PNY1541 and PNY1542 (*AFT1-L99A* integration strains) were designated PNY1543 and PNY1544, for PNY1541, and PNY1545 and PNY1546, for PNY1542.

[0326] Strains were grown in synthetic medium (Yeast Nitrogen Base without Amino Acids (Sigma-Aldrich, St. Louis, MO) and Yeast Synthetic Drop-Out Media Supplement without uracil and histidine (Clontech, Mountain View, CA)) supplemented with 100mM MES pH5.5, 0.2% glucose, and 0.2% ethanol. Overnight cultures were grown in 15 mL of medium in 125 mL vented Erlenmeyer flasks at 30°C, 225 RPM in a New Brunswick Scientific I24 shaker. 18 ml of medium in 125 mL tightly-capped Erlenmeyer flasks was inoculated with overnight culture to an OD_{600} 0.5 and grown for six hours at 30°C, 225 RPM in a New Brunswick Scientific I24 shaker. After six hours, glucose was added to 2.5%, yeast extract was added to 5 g/L, and peptone was added to 10 g/L (time 0 hours). After 24 and 48 hours, culture supernatants (collected using Spin-X centrifuge tube filter units, Costar Cat. No. 8169) were analyzed by HPLC (method described in U.S. Patent Appl. Pub. No. US 2007/0092957, incorporated by reference herein) and LC/MS. Glucose and isobutanol concentrations were determined by HPLC. DHIV was separated and quantified by LC/MS on a Waters (Milford, MA) AcquityTQD system, using an Atlantis T3 (part #186003539) column. The column was maintained at 30°C and the flow

rate was 0.5 mL/min. The A mobile phase was 0.1% formic acid in water, and the B mobile phase was 0.1% formic acid in acetonitrile. Each run consisted of 1 min at 99% A, a linear gradient over 1 min to 25%B, followed by 1 min at 99%A. The column effluent was monitored for peaks at m/z=133 (negative ESI), with cone voltage 32.5V, by Waters ACQ_TQD (s/n QBA688) mass spectometry detector. DHIV typically emerged at 1.2 min. Baseline separation was obtained and peak areas for DHIV were converted to μM DHIV concentrations by reference to analyses of standards solutions made from a 1 M aqueous stock.

[0327] Table 18 shows the DHIV molar yield (moles of DHIV per moles of glucose consumed) and isobutanol titer of the *AFT1-L99A* strains (PNY1543, PNY1544, PNY1545, and PNY1546) and the *FRA2* deletion strain (PNY1506) compared to the parent strain background (PNY1504) at 24 and 48 hours. *AFT1-L99A* expression or the *FRA2* deletion both led to approximately a 50% decrease in the accumulation of DHIV.

Table 18. DHIV molar yield and isobutanol titer.

Strain	24 Hr DHIV Yield (mol/mol)	48 Hr DHIV Yield (mol/mol)	24 Hr Isobutanol Titer (g/L)	48 Hr Isobutanol Titer (g/L)
PNY1504	0.044	0.035	3.7	4.2
PNY1543-PNY1544	0.017	0.015	4.1	5.8
PNY1545-PNY1546	0.019	0.018	4.6	5.5
PNY1506	0.022	0.020	3.8	4.7

Data are the average of two independent flasks, for PNY1504 and PNY1506, and two independent transformants for the *AFT1-L99A* strains (PNY1543-PNY1544 and PNY1545-PNY1546).

[0328] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

Table 12

HMMER2.0 [2.2g] — Program name and version
NAME dhad_for_hmm — Name of the input sequence alignment file
LENG 584 — 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 /app/public/hmmer/current/bin/hmmbuild -F dhad-exp_hmm dhad_for_hmm.aln — Commands used to generate the file: this one means that hmmbuild (default patrameters) was applied to the alignment file
COM /app/public/hmmer/current/bin/hmmcalibrate dhad-exp_hmm — Commands used to generate the file: this one means that hmmcalibrate (default parametrs) was applied to the hmm profile
NSEQ 8 — Number of sequences in the alignment file
DATE Tue Jun 3 10:48:24 2008 — When was the file generated
XT -8455 -4 -1000 -1000 -8455 -4 -8455 -4
NULT -4 -8455 — The transition probability distribution for the null model (single G state).
NULE 595 -1558 85 338 -294 453 -1158 197 249 902 -1085 -142 -21 -313 45 531 201 384 -199 — The symbol emission probability distribution for the null model (G state), consists of K (e.g. 4 or 20) integers. The null probability used to convert these back to model probabilities is 1/K.
EVD -498.650970 0.086142 — The extreme value distribution parameters μ and lambda respectively, both floating point values. Lambda is positive and nonzero. These values are set when the model is calibrated with hmmcalibrate.

HMM	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Position in alignment
	m->m	m->i	m->d	i->m	i->i	d->m	d->d	b->m	m->e												
	-538	*	-1684																		
1(M)	-233	-1296	99	1223	-1477	-1132	89	-1122	420	-1248	1781	1553	-1296	464	-24	-190	-188	-838	-1578	-985	6
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-29	-6203	-7245	-894	-1115	-701	-1378	-538	*												
2(E)	-220	-1288	232	1356	-1807	1016	70	1474	190	-1584	775	132	-1298	300	-282	-183	1140	-1092	-1872	-1262	7
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-29	-6203	-7245	-894	-1115	-701	-1378	*	*												
3(K)	-448	-1932	1558	658	-2220	-1048	40	-1983	1589	-1938	-1091	1558	-1319	450	-193	-278	-419	-1552	-2121	-1397	8
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-29	-6203	-7245	-894	-1115	-701	-1378	*	*												
4(V)	-404	-498	-1497	-939	-588	-1810	-640	1591	914	-127	335	-962	-1866	-562	-767	-868	-357	1720	-1169	-763	9
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-29	-6203	-7245	-894	-1115	-701	-1378	*	*												
5(E)	-265	-1340	-52	1376	-1572	-1189	113	-1125	1345	-1287	-496	99	-1321	595	198	-218	-205	597	-1598	-1032	10
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-29	-6203	-7245	-894	-1115	-701	-1378	*	*												
6(S)	256	-397	-1014	-830	-1841	-646	-862	-1443	-767	1740	-963	-568	-1249	-651	-1007	2267	1586	-862	-2080	-1672	11
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-29	-6203	-7245	-894	-1115	-701	-1378	*	*												
7(M)	-990	-889	-2630	157	-513	-2514	-1346	1309	-1767	820	3693	-1898	-2491	-1496	-1799	-1589	-925	150	-1336	-1041	12
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7105	-8150	-894	-1115	-701	-1378	*	*												

Table 12

8(E)	588	-1875	-194	1628	-2188	-1373	-59	-1931	957	-1890	-977	904	292	393	-162	483	-372	-1495	-2070	-1391	13
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
9(N)	-514	-1116	1207	-315	447	-1650	-304	-778	-224	825	-277	1467	-1738	-123	-618	-627	-454	-803	-1186	783	14
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
10(N)	-815	-1190	-1360	-922	-904	-1967	-797	-442	-670	381	1700	3009	-2099	-654	-934	-1051	-791	-445	-1490	-979	15
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
11(K)	-1530	-2498	-1722	-855	-3141	-2246	-428	-2627	2828	-2404	-1656	-927	662	-2	2047	-1421	-1337	-2324	-2357	-2061	16
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
12(Y)	-872	-1887	-861	-290	-1369	-1801	1662	-1797	325	-1793	-1031	893	-1876	56	2219	-812	-780	-1514	-1565	3287	17
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
13(S)	-830	-1586	-1471	-1099	-2717	-1642	-1010	-2479	-266	-2518	-1746	-1065	-2069	-676	1822	2748	-1000	-1950	-2597	-2189	18
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
14(Q)	-851	-2131	-775	-153	-2554	-1735	-211	-2205	1908	-2094	-1244	-386	-1802	2254	974	1001	-747	-1819	-2181	-1667	19
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
15(T)	-405	-1258	-618	-100	-1490	-1466	1158	-1121	1	-1299	-614	578	-1607	65	-433	960	1849	343	-1677	-1143	20
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
16(I)	-1772	-1325	-4307	-3877	-1405	-3993	-3383	2835	-3705	820	-217	-3632	-3761	-3400	-3682	-3260	-1742	2033	-2838	-2525	21
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
17(T)	-1018	-1329	-2004	-1771	-409	-1993	-1000	-1256	-1512	-1484	-966	-1543	-2367	-1428	-1638	-1257	4050	-1090	-1012	2448	22
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
18(Q)	-1509	-3056	1970	44	-3310	-1666	-896	-3242	-877	-3158	-2439	-322	-2123	3562	-1493	-1259	-1550	-2779	-3260	-2446	23
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
19(D)	-1006	-2199	2378	-88	-3159	1997	-936	-2974	-948	-2977	-2174	-382	-1960	-589	-1571	1295	-1157	-2369	-3178	-2430	24
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											

Table 12

20(M)	445	-796	-1082	-521	-841	-1643	-412	-403	-370	-692	2318	-646	536	1166	-598	-630	660	831	-1204	-767	25
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
21(Q)	741	-990	-1025	-507	-1249	-1551	-519	-720	-357	-1062	-345	-635	-1739	1776	-713	-589	1576	1129	-1559	-1097	26
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
22(R)	-1753	-2648	-2072	-1047	-3365	-2405	-452	-2782	1989	-2495	-1773	-1062	-2379	2402	2843	-1629	-1506	-2504	-2397	-2190	27
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
23(S)	-330	-1010	-1820	-1628	-2778	-1229	-1652	-2481	-1592	-2691	-1841	-1273	2130	-1426	-1834	2449	1034	-1716	-2961	-2594	28
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
24(P)	1882	-1119	-2231	-2302	-3062	-1360	-2209	-2719	-2339	-3013	-2243	-1676	1304	-2117	-2409	-742	-918	-1916	-3263	-3022	29
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
25(N)	969	-1230	-1066	-915	-2593	-1313	-1196	-2242	-1033	-2447	-1626	3157	-1850	-898	-1392	-582	1155	-1644	-2736	-2256	30
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
26(R)	-1847	-2640	-2014	-1161	-3282	-2428	-579	-2818	887	-2553	-1669	-1165	-2462	2447	2838	-1746	-1830	-2555	-2447	-2228	31
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
27(A)	3046	-932	-2480	-2533	-3075	-1200	-2274	-2765	-2501	-3071	-2221	-1658	-1948	-2205	-2512	1225	-739	-1842	-3322	-3078	32
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
28(M)	-2406	-2296	-3638	-3594	-1525	-3105	-2824	-1047	-3121	-596	3018	-3293	-3425	-3046	-2996	-2911	-2552	-1398	-2513	-2207	33
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
29(Y)	-1674	-1506	-2663	-2464	596	-2872	2251	-972	-2024	2197	-552	-1966	-2876	-1730	-1988	-1987	-1601	-1902	-95	2332	34
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
30(Y)	-2013	-2305	-2426	-1781	-328	-2709	-654	-2240	-258	-2064	-1626	-1631	-2788	-899	2789	-2017	-1896	-2130	-857	3434	35
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
31(A)	3822	-1031	-2418	-2539	-3226	1898	-2364	-2941	-2626	-3229	-2379	-1722	-2026	-2302	-2634	-654	-848	-1983	-3415	-3226	36
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

32(I)	-1247	-941	-3589	-3639	-1082	-3101	-2185	-2227	-2763	766	-76	-2703	-3050	-2469	-2697	-2253	1322	1974	-1985	-1633	37
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
33(G)	-2594	-2690	-3304	-3623	-4323	-3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	38
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
34(F)	-1511	-1236	-3511	-3017	-2747	-2982	-1069	-260	-2651	992	2737	-2407	-2904	-2088	-2418	-2099	-1434	-489	-537	2056	39
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
35(Q)	-576	-1669	-401	92	-2232	831	-173	-1930	1505	-1913	-1042	-166	-1620	1653	-51	-482	1346	-1534	-2098	-1490	40
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
36(D)	-1352	-3066	3025	1349	-3303	-1566	-724	-3141	1155	-3043	-2267	-165	-1991	-354	-1350	-1086	-1368	-2659	-3221	-2356	41
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
37(E)	-1507	-3288	2042	2782	-3520	515	-853	-3401	-981	-3296	-2566	-182	-2064	-503	-1753	-1209	-1553	-2895	-3486	-2547	42
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
38(D)	-1445	-2778	3529	-53	-3524	-1590	-1129	-3476	-1367	-3459	-2774	-396	-2156	-525	-2122	554	-1609	-2880	-3582	-2717	43
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
39(F)	-2658	-2176	-4219	-4000	-3881	-3933	-1352	-531	-3633	1121	-19	-3184	-3709	-2820	-3296	-3219	-2579	-1637	-601	403	44
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
40(D)	-684	-2193	1738	1460	-2494	-1437	-249	-2257	1694	-2199	-1308	-62	-1637	185	-450	-531	633	-1808	-2374	-1657	45
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
41(K)	-2620	-2061	-2461	-2046	-3743	-2791	-1570	-3603	-3284	-3387	-2939	-2048	-3039	-1260	-465	-2604	-2536	-3331	-3001	-2988	46
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
42(P)	1882	-1119	-2231	-2302	-3062	-1360	-2209	-2710	-2339	-3013	-2243	-1676	3304	-2117	-2409	-742	-918	-1816	-3263	-3022	47
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
43(I)	-1006	-992	-2347	-1784	-650	-2452	-1256	-2372	-1386	77	2213	-1720	-2455	2030	-1490	-1528	-946	106	-1441	-1111	48
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

44(V)	-1771	-1603	-3750	-3689	-2037	-3050	-3231	403	-3479	-1154	-1076	-3246	-3399	-3363	-3437	-2828	-1917	-3688	-3074	-2677	49
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
45(G)	-2584	-2690	-3304	-3623	-4328	3748	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	50
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
46(I)	-1759	-1303	-4330	-3968	-1751	-4051	-3743	3027	-3837	-597	-528	-3729	-3675	-3688	-3910	-3369	-1751	2438	-3259	-2819	51
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
47(V)	1736	-1012	-3546	-3678	-1377	-3075	-2434	2052	-2843	-608	-331	-2754	-3122	-2619	-2855	-2270	-1277	2188	-2333	-1941	52
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
48(N)	-686	-1511	-702	-806	-2027	-1386	-1939	-2841	-1264	-2950	-2137	2408	-1979	-1062	-1648	2444	-971	-2105	-3054	-2475	53
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
49(M)	-411	-857	-1800	-1434	-1528	1914	-1202	-1029	-1247	-1347	2909	-1217	-1912	-1119	-1444	-676	1550	-767	-1922	-1539	54
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
50(W)	-782	-1258	793	-883	1193	346	2051	-932	-556	-1092	-441	-798	-1993	-426	-909	-904	-720	-779	3168	1546	55
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
51(W)	1009	-798	-1470	-935	-463	-1773	-545	-460	-751	-736	-66	-943	-1904	-606	-1002	1604	-507	-322	2818	1521	56
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
52(D)	-1137	-2711	2125	1647	-2995	-1523	-617	-2786	-528	-2743	-1933	-150	-1897	-234	-1165	-924	2117	-2331	-2948	-2141	57
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
53(I)	-590	-1102	-1031	-829	-1522	1429	-927	2119	-880	-1369	-699	1602	-1038	-750	-1186	-799	-698	-689	-1887	-1419	58
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
54(T)	-666	-1412	-954	-984	-2702	-1428	-1357	-2418	-1208	-2650	-1886	2293	-2000	-1101	-1519	-787	2967	-1835	-2866	-2360	59
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
55(P)	-632	-1230	-2074	-2144	-2996	-1453	-2116	-2631	-2128	-2928	-2213	-1658	3610	-2006	-2221	-852	1302	-1931	-3185	-2917	60
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

56(C)	-2476	6736	-4102	-4358	-3712	-2763	-3545	-3518	-4167	3859	-3569	-3631	-3363	-4030	3832	-2793	-2860	-3158	-3464	-3718	61
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
57(N)	-2171	-2655	-1458	-1748	-3334	-2364	-2267	-3943	-2365	-3936	-3437	4206	-2932	-2205	-2608	-2224	-2439	-3392	-3253	-2909	62
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
58(M)	672	-918	-3119	-2578	-742	-2866	-1734	1807	-2263	16	3713	-2271	-2704	-1960	-2216	-1806	-1058	493	-1612	-1306	63
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
59(H)	-1525	-2164	-1235	-1346	-2509	2296	4239	-3172	-1516	-3178	-2523	-1448	-2541	-1520	-1760	-1591	-1741	-2656	-2681	-2065	64
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
60(L)	-2478	-2009	-4717	-1196	-568	-4424	-3262	1334	-3887	2828	604	-4085	-3872	-3088	-3590	-3717	-2380	-199	-2217	-2207	65
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
61(H)	-682	-2191	1015	275	-2485	396	2379	-2251	62	-2197	-1307	1826	-1636	1527	-480	-529	-641	-1803	-2375	-1654	66
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
62(D)	-575	-1920	1978	184	-2299	94	-242	-2029	114	-2025	-1144	-126	-1608	186	1063	-469	1413	-1605	-2229	-1561	67
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
63(L)	-2618	-2139	-4597	-4163	2144	-4285	-2934	-83	-3854	2980	638	-3771	-3800	-2950	-3483	-3503	-2005	-751	-1442	-808	68
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
64(A)	3662	-1033	-2408	-2532	-3233	2193	-2364	-2950	-2626	-3237	-2386	-1719	-2027	-2301	-2635	-655	-850	-1988	-3420	-3231	69
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
65(K)	-443	-1857	958	270	-2158	-1393	-66	-1890	1839	-442	-957	-36	-1499	1204	-132	616	-382	-1469	-2048	-1383	70
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
66(C)	605	1553	739	-17	-1374	-1488	-182	260	969	-203	-397	-263	-1573	159	891	-426	-331	-761	-1567	-1032	71
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
67(A)	3327	-956	-3193	-2728	-1289	-2677	-2114	1664	-2485	-601	-288	-2403	-2839	-2263	-2523	-1871	-1126	1617	-2143	-1765	72
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

68(K)	-532	-1656	-490	1321	-1891	-1527	-172	-124	2206	-1591	-782	-223	-1619	237	-106	-482	-464	-98	-1904	-1326	73
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
69(H)	384	-1854	936	889	-2165	-1363	1438	-1909	1111	-1866	-948	1091	-1464	421	-131	-284	-342	-69	-2043	-1364	74
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
70(G)	1823	-932	-2330	-2313	-3120	2511	-2158	-2865	-2331	-3098	-2209	-1563	-1912	-2032	-2419	1138	-706	-1883	-3328	-3077	75
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
71(V)	-1760	-1333	-4244	-3769	-1262	-3902	-3190	1495	-3568	1270	-96	-3536	-3677	-3238	-3534	-3148	-1725	2665	-2654	-2373	76
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
72(W)	-1054	-2172	-1112	-403	-2566	-1917	-286	-2196	2516	-2095	-1292	1183	-1958	140	1333	-959	-922	-1867	2591	-1720	77
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
73(D)	611	-1995	1525	937	-2295	-1400	-148	-2043	211	-2006	-1106	-37	-1553	1420	-312	-408	1235	-1609	-2193	-1499	78
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
74(A)	2716	-902	-2380	-2205	-2799	-1197	-1975	-2459	-2081	-2736	-1895	-1520	-1895	-1844	-2201	1191	1299	-1669	-3045	-2756	79
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
75(G)	-1709	-2633	2424	-409	-3781	2819	-1457	-3777	-1728	-3733	-3076	-739	-2989	-1180	-2441	-1557	-1893	-3158	-3660	-3038	80
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-212	-2909	-8150	-273	-2534	-701	-1378	*		*											
76(A)	2528	-1119	-2614	-2330	-1245	-1983	-1829	-377	-2042	1435	-341	-1937	-2411	-1873	-2088	-1266	-1059	-397	-2063	-1713	82
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
77(W)	-472	-361	-2421	-1812	-298	-1979	-826	1164	-1486	-143	2485	373	-2028	-1185	-1426	-1048	-412	1116	2998	-454	83
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
78(P)	-1198	-1737	-2187	-2394	-3665	2006	-2550	-3630	-2743	-3756	-3008	-2052	3474	-2495	-2835	-1401	-1593	-2736	-3511	-3519	84
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
79(Q)	-999	-1075	-2106	-1568	-726	-2370	-1175	83	-1185	1373	218	-1566	-2400	2445	-1340	-1445	-946	1441	-1501	-1146	85
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											

Table 12

80(Q)	-885	-779	-2609	-2618	-481	-2414	-1253	1645	-1736	799	1924	-1827	-2405	2252	-1752	-1484	-621	802	-1240	-935	86
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
81(F)	-3342	-2776	-4026	-4232	4394	-3545	-1431	-2315	-4038	-1801	-1900	-3299	-3780	-3350	-3645	-3490	-3420	-2566	-759	349	87
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
82(G)	-998	-2100	-120	-175	-2567	2528	2174	-2558	-587	-2583	-1806	1422	-1966	-461	-1038	-925	-1088	-2095	-2657	-1948	88
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
83(T)	-1213	-1674	-2755	-2906	-3163	-1922	-2659	-2698	-2758	-3105	-2612	-2311	-2600	-2708	-2753	-1463	3819	-2197	-3286	-3156	89
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
84(I)	-1286	-1279	-2907	-3683	-1446	-2549	-2198	4291	-2407	-726	-534	-2386	1172	-2299	-2437	-1895	-1392	283	-2302	-1913	90
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
85(T)	-493	-1105	-2189	-2267	-3101	1880	-2196	-2791	-2334	-3081	-2269	-1649	-2058	-2099	-2410	-719	3135	-1948	-3282	-3046	91
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
86(V)	-1750	-1296	-4319	-3957	-1765	-4038	-3733	2364	-3826	-619	-543	-3716	-3869	-3685	-3902	-3354	-1743	3012	-3265	-2817	92
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
87(S)	923	-962	-2348	-2422	-3132	-1207	-2248	-2850	-2440	-3140	-2285	-1024	-1954	-2158	-2477	3171	-758	-1896	-3362	-3103	93
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
88(D)	-2784	-3432	1016	-1200	-4140	-2466	-2197	-4505	-2621	-4365	-3956	-1551	-3014	-2039	-3232	-2593	-2938	-4046	-3710	-3552	94
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
89(G)	-2594	-2690	-3304	-3623	-4328	3775	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	95
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
90(I)	-1880	-1493	-4193	-3724	-953	-3837	-2960	5251	-3420	257	2372	-3485	-3608	-3005	-3310	-3087	-1840	617	-2373	-2155	96
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
91(S)	2150	-939	-2407	-2415	-3075	-1197	-2205	-2781	-2384	-3085	-2205	-1613	-1938	-2105	-2436	2652	-729	-1850	-3306	-3049	97
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

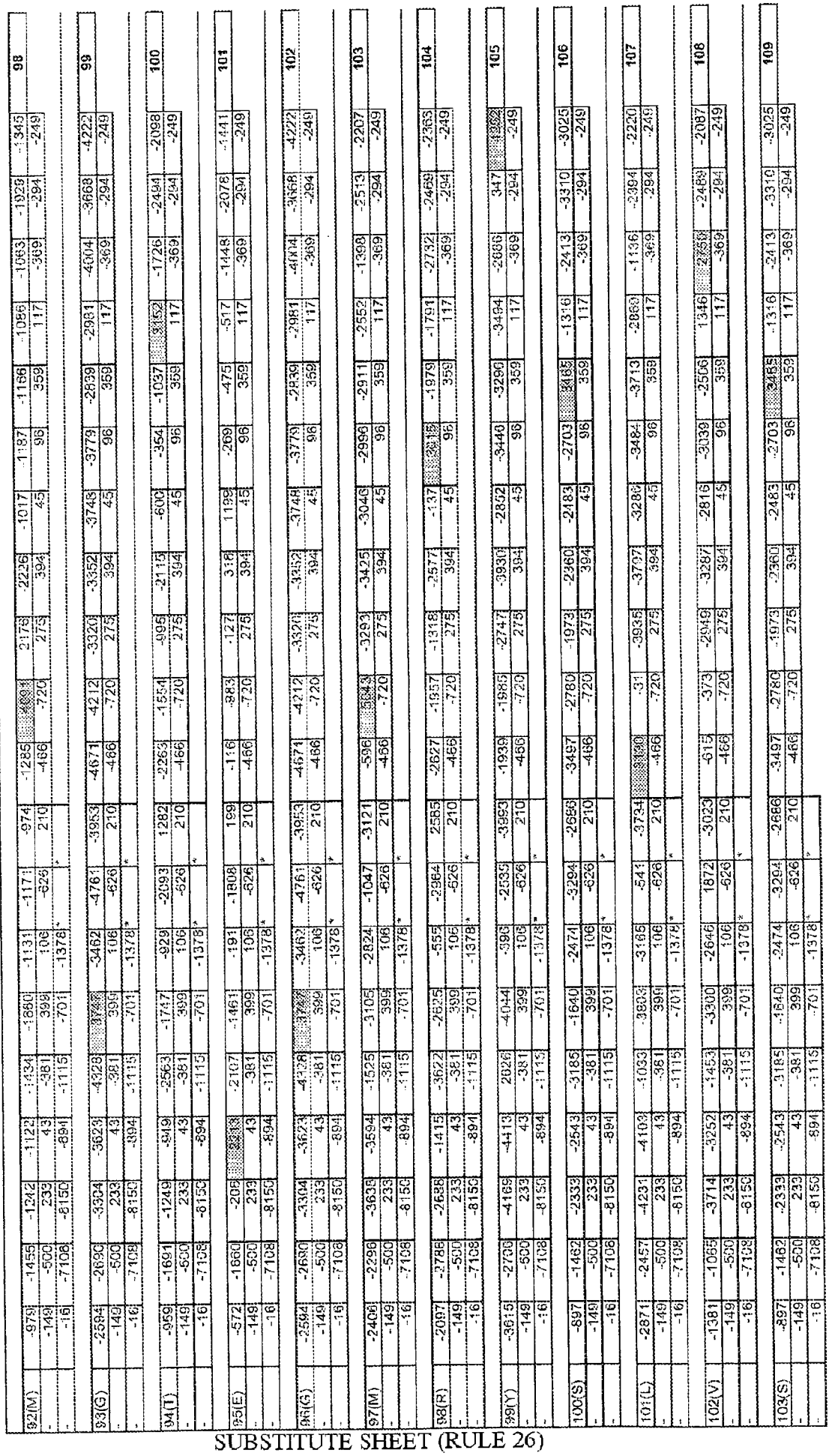

Table 12

104(R)	-2957	-3022	-3318	-2735	-3796	-2998	-1968	-3912	-646	-3631	-3157	-2611	-3280	-1724	-4856	-3026	-2913	-3650	-3096	-3185	110
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
105(E)	-1719	-3572	-2596	-2779	-3767	-1632	-993	-3700	-1241	-3578	-2920	-234	-2167	-666	-2090	-1360	-1789	-3182	-3742	-2756	111
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
106(V)	-1746	-1296	-4308	-3846	-1757	-4020	-3712	2190	-3811	-614	-539	-3702	-3858	-3667	-3884	-3396	-1740	-3098	-3250	-2803	112
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
107(I)	-2091	-1746	-3971	-3840	-1676	-3532	-3289	-3884	-3581	-659	-693	-3562	-3674	-3445	-3521	-3194	-2146	449	-2877	-2493	113
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
108(A)	-3438	-1472	-2845	-3040	-3287	-1726	-2735	-2840	-3028	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1367	-2183	-3405	-3320	114
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
109(D)	-2784	-3432	-4015	-1200	-4140	-2466	-2197	-4505	-2621	-4365	-3956	-1551	-3014	-2039	-3232	-2593	-2938	-4046	-3710	-3552	115
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
110(S)	-352	2942	-2955	-2957	-2878	-1254	-2382	-2573	-2892	-2927	-2128	-1827	-2001	-2405	-2607	-8103	-778	-1757	-3171	-2911	116
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
111(I)	-2091	-1746	-3971	-3840	-1676	-3532	-3239	-3884	-3581	-659	-693	-3562	-3674	-3446	-3521	-3194	-2146	449	-2877	-2493	117
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
112(E)	-2641	-3308	-896	-3722	-3966	-2458	-2043	-4105	-2128	-4016	-3555	-1531	-2959	-1842	-2560	-2479	-2750	-3722	-3563	-3385	118
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
113(T)	1556	-936	-2493	-2457	-2805	-1256	-2159	-2210	-2319	-2681	-1932	-1656	-1874	-2089	-2352	-598	-3238	-1547	-3111	-2847	119
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
114(C)	1784	-2118	-2013	-1532	-1093	-1580	-1089	-436	-1322	-937	-273	1093	-1932	-1127	-1472	-748	-515	1585	-1536	-1163	120
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
115(M)	1831	2019	-2596	-2038	-605	-1979	-1126	244	-1727	-359	-2501	-1655	-2145	-1435	-1683	-1106	-557	1087	-1153	-804	121
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

116(G)	-987	-2211	-43	-62	-2833	2229	-691	-2816	-407	-2604	-1797	1197	-1917	2280	-858	-880	-1045	-2139	-2772	-2099	122
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
117(G)	2313	-1042	-2391	-2526	-3250	2621	-2372	-2972	-2637	-3257	-2407	-1721	-2032	-2310	-2646	-662	-859	-2003	-3454	-3247	123
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
118(Q)	-914	-2350	-48	1661	-2621	-1571	2504	-2400	68	-2331	-1486	-201	-1796	2846	-351	-754	-865	-1984	-2463	-1787	124
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
119(W)	-517	-1294	-733	-183	-1062	-1605	-234	-1037	19	-1207	-456	1455	-1690	33	756	411	-454	-619	3846	1266	125
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
120(M)	410	-469	-2417	-1828	-341	-2041	-897	195	-1513	-156	3130	-1534	-2102	-1230	-1484	-1117	-507	954	-894	2253	126
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
121(D)	-2784	-3432	4015	-1209	-4140	-2466	-2197	-4505	-2621	-4365	-3956	-1551	-3014	-2039	-3232	-2593	-2938	-4046	-3710	-3552	127
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
122(G)	2142	-930	-2334	-2298	-3160	2237	-2139	-2842	-2302	-3074	-2187	-1557	-1909	-2010	-2397	1138	-701	-1871	-3308	-3053	128
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
123(V)	-1514	-1144	-3950	-3459	1821	-3487	-2577	2274	-3208	-209	-87	-3112	-3362	-2864	-3113	-2680	-1476	2428	-2194	-1766	129
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
124(V)	-1743	-1294	-4292	-3873	-1511	-3988	-3433	2287	-3712	598	-319	-3626	-3774	-3456	-3716	-3260	-1717	2798	-2931	-2577	130
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
125(A)	6911	-654	-2608	-2665	-2116	-1577	-2106	-575	-2445	-1646	-1202	-1906	-2208	-2218	-2451	-901	-876	1294	-2727	-2394	131
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
126(I)	-1764	-1323	-4298	-3936	-1668	-3994	-3655	3337	-3783	-508	-462	-3689	-3838	-3608	-3835	-3311	-1759	1847	-3164	-2747	132
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
127(G)	-1157	-1705	-2169	-2375	-3654	3021	-2534	-3611	-2730	-3741	-2984	-2024	2418	-2475	-2826	-1361	-1555	-2705	-3513	-3509	133
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

128(G)	-2594	-2690	-3304	-3623	-4328	376	-3462	-4761	-3953	-4671	-4212	-3920	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	134
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
129(C)	-2476		-4102	-4358	-3712	-2763	-3545	-3518	-4167	-3859	-3569	-3631	-3363	-4030	-3832	-2793	-2860	-3158	-3464	-3718	135
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
130(D)	-2784	-3432	1018	-1209	-4140	-2466	-2197	-4505	-2621	-4365	-3956	-1551	-3014	-2039	-3232	-2593	-2938	-4046	-3710	-3552	136
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
131(K)	-2620	-2961	-2461	-2048	-3743	-2791	-1570	-3603	3784	-3387	-2839	-2048	-3039	-1260	-465	-2604	-2536	-3331	-3001	-2968	137
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
132(N)	-2171	-2655	-1456	-1748	-3934	-2364	-2267	-3943	-2365	-3936	-3437	1208	-2932	-2205	-2608	-2224	-2439	-3392	-3253	-2909	138
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
133(M)	-2406	-2296	-3638	-3594	-1525	-3105	-2824	-1047	-3121	-596	5043	-3293	-3425	-3046	-2996	-2911	-2552	-1398	-2513	-2207	139
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
134(P)	-2931	-2878	-3420	-3708	-4151	-2925	-3468	-4621	-3859	-4490	-4165	-3491	4228	-3781	-3695	-3182	-3279	-4087	-3594	-4064	140
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
135(G)	-2594	-2690	-3304	-3623	-4328	376	-3462	-4761	-3953	-4671	-4212	-3920	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	141
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
136(A)	2180	-935	-2286	-2196	-3057	1098	-2058	-2796	-2174	-3021	-2134	-1516	-1898	-1906	-2302	2146	-689	-1849	-3256	-2983	142
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
137(M)	-1790	-1433	-4142	-3579	-669	-3668	-2608	1558	-3293	1235	3998	-3296	-3401	-2717	-3088	-2843	-1726	1156	-3002	-1868	143
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
138(I)	-2091	-1746	-3971	-3840	-1676	-3532	-3289	3884	-3581	-659	-693	-3562	-3674	-3445	-3521	-3194	-2146	449	-2877	-2493	144
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
139(A)	3103	-1036	-2445	-2572	-3222	1051	-2380	-2930	-2650	-3226	-2381	-1739	-2034	-2327	-2648	-664	-857	-1981	-3412	-3228	145
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											

Table 12

140(M)	-2325	-1931	-4598	-4612	-498	-4222	-3013	-1242	-3722	-1864	-3858	-3711	-2810	-3414	-3439	-2215	-299	-2075	-2098	146					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	394	45	96	359	117	-369	-294	-249						
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
141(A)	-1036	-2445	-2572	-3222	1551	-2380	-2930	-2650	-3226	-2381	-1739	-2034	-2327	-2648	-664	-857	-1981	-3412	-3228	147					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
142(R)	-1588	-2442	-1399	-953	-3069	-2171	-709	-2795	373	-2625	-1916	1858	-2357	-324	-1520	-1505	-2453	-2523	-2186	148					
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
143(M)	-1446	-1256	-3396	-2618	-474	-3024	-1923	175	-2473	2225	2666	-2574	-2922	-2063	-2375	-2153	852	151	-1566	-1410	149				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
144(N)	-1662	-3326	2055	78	-3621	-1643	-1941	-3622	-1272	-3531	-2870	3677	-2182	-724	-2571	-1371	-1757	-3693	-3633	-2791	150				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
145(I)	-1066	-921	-2825	-2239	-1041	-2675	-1601	3335	-1668	-455	-92	-2087	-2692	-1688	1701	-1795	-1024	1960	-1771	-1396	151				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
146(P)	-2931	-2878	-3422	-3706	-4181	-2925	-3468	-4621	-3859	-4490	-4165	-3491	4228	-3781	-3895	-3182	-3278	-4087	-3594	-4064	152				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
147(S)	1568	-940	-2267	-2192	-3082	1101	-2068	-2826	-2165	-3048	-2159	-1615	-1901	-1915	-2313	2603	-694	-1866	-3279	-3000	153				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
148(I)	-1880	-1492	-4195	-3728	-963	-3841	-2981	3272	-3425	246	2277	-3490	-3613	-3014	-3317	-3092	-1841	628	-2385	-2163	154				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
149(F)	-2204	-1797	-3724	-3478	3308	-3383	-628	-1077	-3002	-746	3167	-2502	-3309	-2372	-2702	-2535	-2120	-1245	28	2460	155				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
150(V)	1265	-1028	-3200	-2994	-1833	-2150	-2460	417	-2771	-1122	-818	-2348	-2640	-2539	-2766	-1464	-1118	3028	-2700	-2325	156				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																
-																									
151(Y)	-3462	-2868	-3701	-3919	238	-3552	-1112	-3000	-3638	-2516	-2526	-3027	-3773	-3101	-3341	-3418	-3527	-3071	-441	4733	157				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249					
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*																

Table 12

152(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	158
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
153(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	159
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
154(T)	-359	-976	-2225	-2229	-2900	-1242	-2074	-2560	-2170	-2875	-2064	-1561	-1958	-1969	-2247	1110	3375	-1760	-3152	-2850	160
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
155(I)	-2091	-1746	-3971	-3840	-1676	-3532	-3289	3684	-3561	-659	-693	-3562	-3674	-3445	-3521	-3194	-2146	449	-2877	-2493	161
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
156(H)	861	-1924	-384	1610	-2260	-1477	1787	-1974	1769	-1918	-1022	-120	-1566	362	697	-417	-459	-1557	-2073	-1446	162
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
157(P)	-655	-1502	-711	-557	-2204	-1463	2143	-2122	-586	-2233	-1445	-686	2341	-560	-941	855	-805	-1657	-2369	-1763	163
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
158(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	164
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
159(H)	-744	-2193	-114	1118	-2513	-1612	2456	-2252	1178	-2183	-1306	2230	-1889	180	-233	-598	-687	-1823	-2339	-1670	165
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
160(W)	-2672	-2139	-3850	-3748	941	-3611	-469	-1691	-3306	1047	-1217	-2551	-3534	-2514	-2960	-2788	-2577	-1799	1295	3466	166
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
161(K)	386	-1981	779	279	-2295	-1403	-114	-2043	2289	-1991	-1082	941	-1536	1289	-211	-384	-457	-1602	-2161	-1476	167
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
162(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	168
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
163(K)	-1144	-2365	-912	2048	-2856	-1912	-328	-2459	2267	-2295	-1482	-556	-1989	108	1334	-1013	-1014	-2093	-2324	-1881	169
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

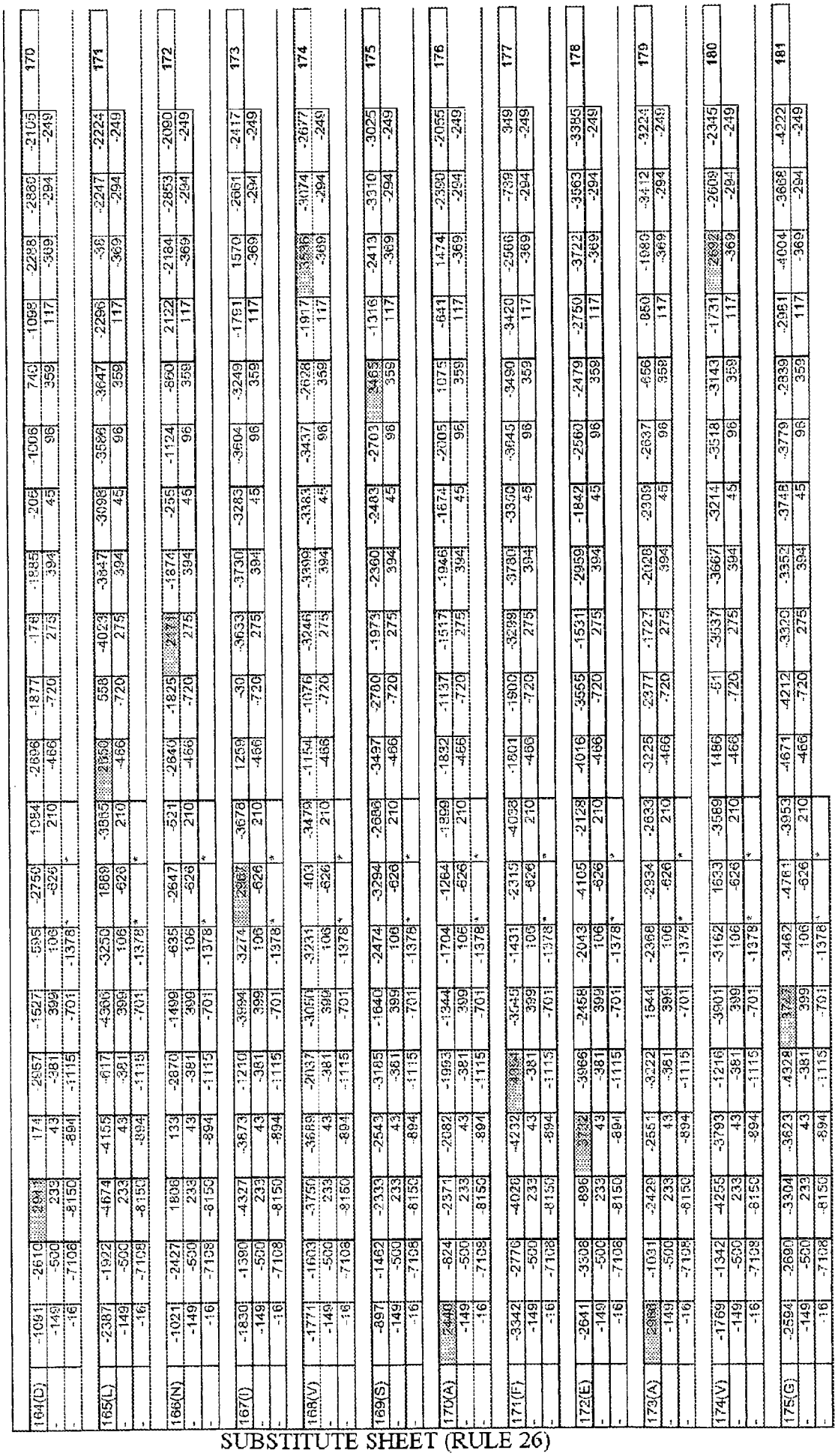

Table 12

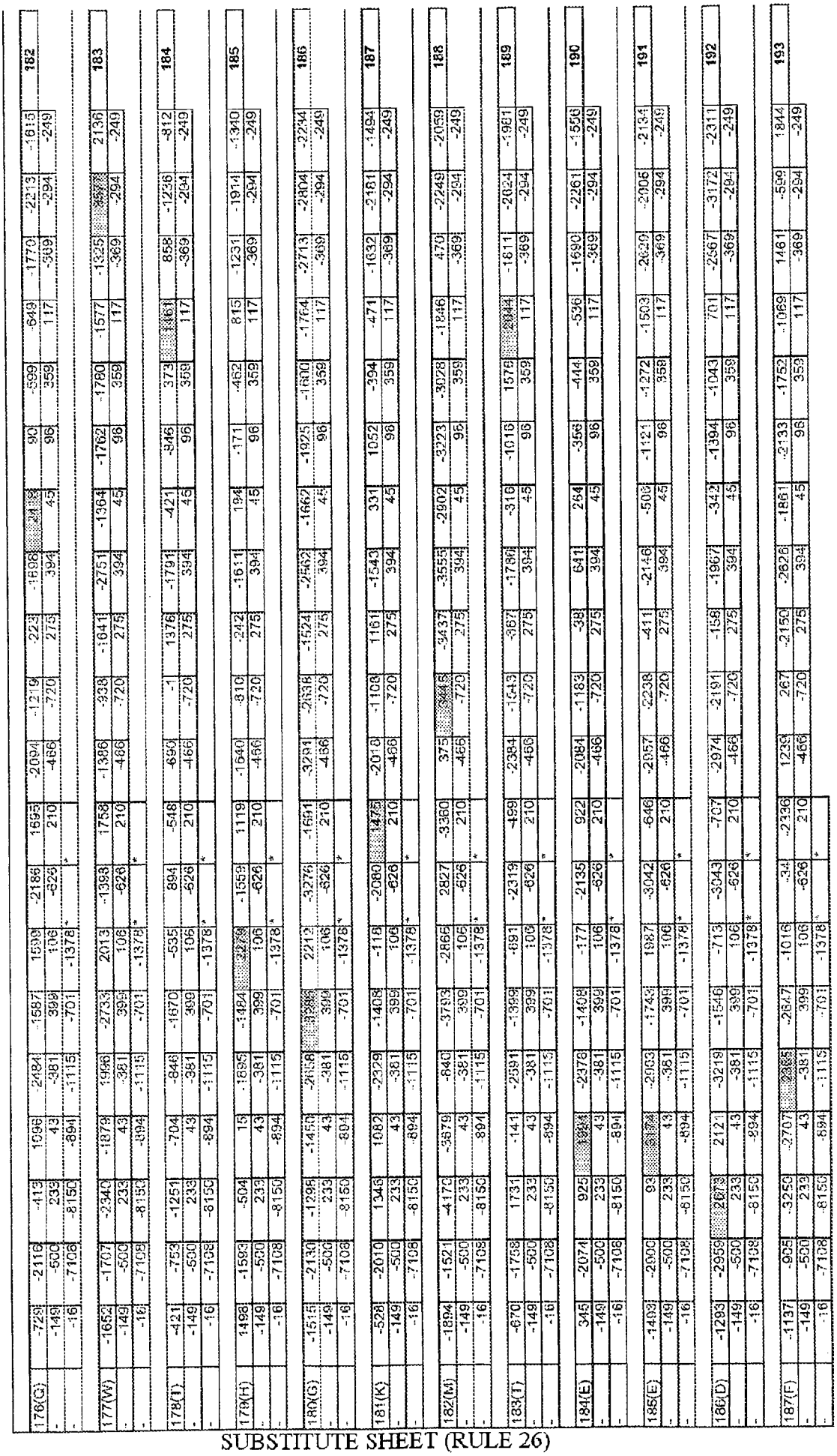

Table 12

188(K)	-479	-1713	-409	1031	-1925	-1457	1755	-1650	1844	-349	-827	-140	-1556	319	-75	-403	-411	-1301	-1900	843	194
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
189(G)	433	-2144	52	1047	-2717	2303	-615	-2467	-442	-2462	-1655	1123	-1828	-233	-995	-763	-923	-2000	-2716	-2005	195
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
190(V)	-1752	-1320	-4251	-3806	-1311	-3916	-3232	1701	-3614	1188	-140	-3551	-3693	-3280	-3568	-3166	-1718	2833	-2703	-2409	196
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
191(E)	-1189	-1750	-734	2668	-1820	-2036	-1068	1892	-867	-1273	-897	-922	-2295	-797	-1238	-1340	-1167	-426	-2325	-1789	197
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
192(C)	-1182	3528	-1398	-620	-2541	-2038	-358	-2093	1181	-2037	-1272	-747	-2070	1553	2213	-1123	-1038	-1817	-2142	-1774	198
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
193(N)	-1478	-2527	-261	-403	-2011	-1637	2032	-2925	-735	-2845	-2195	3635	-2259	-721	-1085	-1352	-1546	-2522	-2307	-1431	199
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
194(A)	3436	-1472	-2846	-3040	-3287	-1726	-2735	-2840	-3028	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	200
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
195(C)	-1220	4911	-3609	-3314	-1440	-2025	-2482	1565	-2822	-706	-544	-2678	-2896	-2710	-2830	-1809	-1375	379	-2371	-1957	201
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
196(P)	-2931	-2878	-3420	-3706	-4181	-2925	-3468	-4621	-3859	-4490	-4165	-3491	4226	-3781	-3695	-3182	-3279	-4087	-3594	-4064	202
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
197(G)	-477	-1115	-1983	-2189	-3315	1154	-2272	-3172	-2506	-3387	-2522	-1599	-2042	-2177	-2583	1217	-905	-2130	-3477	-3225	203
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
198(A)	1653	-1347	-705	-249	-1969	-1385	-477	-1629	-159	-1759	-935	-434	1285	1404	-586	-450	1019	-1243	-2070	-1522	204
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
199(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	205
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

200(S)	1870	-938	-2270	-2183	-3068	1488	-2056	-2810	-2168	-3032	-2144	-1511	-1898	-1901	-2300	2236	-690	-1857	-3265	-2980	206
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
201(C)	-2476	[illegible]	-4102	-4358	-3712	-2763	-3545	-3513	-4167	-3859	-3569	-3631	-3363	-4030	-3832	-2793	-2860	-3158	-3464	-3718	207
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
202(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	208
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
203(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	209
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
204(M)	-2406	-2286	-3638	-3594	-1525	-3105	-2824	-1047	-3121	-596	1048	-3293	-3425	-3046	-2996	-2911	-2552	-1398	-2513	-2207	210
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
205(Y)	-3590	-2700	-4146	-4379	2092	-4028	-404	-2517	-3963	-1928	-1973	-2744	-3921	-2845	-3431	-3284	-3474	-2669	336	4423	211
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
206(T)	-1213	-1674	-2755	-2906	-3163	-1922	-2659	-2693	-2788	-3105	-2612	-2311	-2600	-2708	-2753	-1463	3819	-2197	-3286	-3156	212
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
207(A)	3438	-1472	-2846	-3040	-3287	-1726	-2730	-2840	-3028	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	213
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
208(N)	-2171	-2655	-1458	-1748	-3334	-2364	-2267	-3943	-2365	-3936	-3437	4205	-2932	-2205	-2608	-2224	-2439	-3392	-3253	-2909	214
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
209(T)	-1213	-1674	-2755	-2906	-3163	-1922	-2659	-2693	-2788	-3105	-2612	-2311	-2600	-2708	-2753	-1463	3819	-2197	-3286	-3156	215
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
210(M)	-2355	-1988	-4343	-3834	-504	-4051	-2868	105	-3385	1451	4880	-3680	-3671	-2806	-3171	-3327	-2274	-474	-2039	-1925	216
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
211(S)	2150	-939	-2407	-2415	-3075	-1197	-2205	-2781	-2384	-3065	-2205	-1613	-1936	-2105	-2436	2652	-729	-1850	-3306	-3049	217
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

212(S)	-344	-979	-2190	-2162	-2959	-1227	-2042	-2651	-2116	-2934	-2100	-1528	-1941	-1909	-2222	-2218	1775	-1804	-3187	-2882	218
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
213(A)	-3048	-932	-2480	-2533	-3075	-1200	-2274	-2765	-2501	-3071	-2221	-1658	-1948	-2205	-2512	1225	-739	-1842	-3322	-3078	219
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
214(I)	-1924	-1546	-4067	-3658	2312	-3663	-2081	3033	-3367	150	99	-3197	-3492	-2821	-3179	-2894	-1877	293	-1445	-692	220
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
215(E)	-2641	-3308	-896	3732	-3966	-2458	-2043	-4105	-2128	-4016	-3555	-1531	-2959	-1842	-2560	-2479	-2750	-3722	-3563	-3385	221
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
216(A)	-3048	-814	-2506	-2162	-1896	-1545	-1698	-499	-1942	-1398	-813	-1640	-2076	-1723	-2027	-806	1148	1559	-2200	-1856	222
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
217(M)	-2576	-2118	-4725	-4165	-461	-4430	-3165	99	-3811	2513	3454	-4075	-3839	-2978	-3488	-3704	-2457	-591	-2111	-2145	223
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
218(G)	-2594	-2690	-3304	-3823	-4328	3743	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	224
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
219(M)	-2313	-1968	-4256	-3765	-518	-3960	-2800	96	-3269	1292	4228	-3089	-3630	-2769	-3097	-3249	-2243	-457	-2026	-1874	225
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
220(S)	-897	-1462	-2333	-2543	-3185	-1640	-2474	-3294	-2686	-3497	-2780	-1973	-2360	-2483	-2703	3465	-1316	-2413	-3310	-3025	226
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
221(L)	-2631	-2159	-4786	-4228	-462	-4506	-3231	96	-3878	2529	2482	-4157	-3880	-3016	-3541	-3793	-2509	-608	-2134	-2182	227
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
222(P)	-1501	-1778	-2473	-2371	-1710	-2311	-2045	-1321	-2060	827	-1068	-2173	3594	-2082	-2130	-1799	-1699	-1373	-2373	-1942	228
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
223(Y)	-1068	-1670	-865	-836	-631	1198	-767	-1828	-1059	-1914	-1304	692	-2203	-906	-1387	-1136	-1163	-1566	-1185	3870	229
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											

Table 12

224(S)	-897	-1462	-2333	-2543	-3185	-1640	-2474	-3294	-2656	-3497	-2780	-1973	-2360	-2483	-2703	-3488	-1316	-2413	-3315	-3025	230			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
225(S)	1172	-954	-2367	-2422	-3120	-1204	-2237	-2835	-2426	-3122	-2265	-1621	-1946	-2145	-2467	-3107	-749	-1864	-3349	-3092	231			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
226(S)	-342	-975	-2176	-2124	-2912	-1229	-2003	-2594	-2067	-2878	-2048	-1510	-1936	-1866	-2184	-2553	2492	-1773	-3143	-2833	232			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
227(M)	-720	-1440	-713	-343	-1228	-1693	2436	-1209	-132	-1364	3669	1904	-1652	-183	-458	-776	-660	-1604	-1545	-890	233			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
228(P)	2240	-1100	-2241	-2293	-3037	-1346	-2188	-2663	-2317	-2986	-2210	-1663	3031	-2093	-2391	-732	-885	-1893	-3243	-2998	234			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
229(A)	2958	-1235	-1299	-1377	-2868	-1345	-1673	-2580	-1661	-2843	-2054	1555	-1995	-1468	-1921	-715	-888	-1871	-3064	-2630	235			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
230(E)	-509	-1046	-834	1564	-1116	-1669	-441	-465	-283	250	-206	-577	689	-200	-856	-670	-459	1290	-1467	-595	236			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
231(D)	-1203	-2412	2998	-117	-3286	-1536	-1057	-3176	-1163	-3186	-2436	-428	-2088	-730	-1824	2377	-1368	-2578	-3334	-2552	237			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
232(Q)	954	-1983	-100	971	-2337	177	-267	-2067	81	-2060	-1189	-125	-1637	2699	-418	-514	-597	-1649	-2268	-1597	238			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
233(E)	-2641	-3308	-696	3552	-3066	-2456	-2043	-4105	-2128	-4016	-3555	-1531	-2659	-1842	-2560	-2479	-2750	-3722	-3563	-3385	239			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
234(K)	-2620	-2961	-2461	-2046	-3743	-2731	-1570	-3603	3764	-3367	-2839	-2048	-3039	-1260	-465	-2604	-2536	-3331	-3601	-2986	240			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
235(R)	377	-1802	-415	988	-2095	-1474	-95	-1786	1452	-1785	-911	-135	-1560	343	1555	-409	-431	376	-1986	-1375	241			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																

Table 12

236(D)	1083	-1585	2682	-244	-1941	-1573	-679	812	-527	-1651	-980	-490	-1869	-358	-1003	-771	-766	-903	-2208	-1633	242
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
237(E)	-1225	-2868	1694	1948	-3149	-1532	-671	-2375	-630	-2902	-2101	-150	-1935	-293	-1299	1884	-1241	-2496	-3093	-2248	243
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
238(C)	1375	3262	-2620	-2108	-827	-1866	-1267	1631	-1811	-599	-10	-1674	-2137	-1531	-1786	-1034	790	249	-1361	-1010	244
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
239(E)	635	-1796	1055	1761	-2018	-1464	-263	1191	28	-1767	-846	-148	-1637	135	-481	-520	-553	-1300	-2077	-1441	245
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
240(E)	593	-2044	-252	2548	-2437	-1542	-329	-2133	151	-2120	-1274	-244	-1738	89	946	-646	-717	-1734	-2305	-1686	246
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
241(S)	1884	-835	-1962	-1576	-1634	-1436	-1320	1041	-1409	-1453	-781	-1293	-1922	-1241	-1606	1373	-597	-669	-2036	-1656	247
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
242(G)	2267	-1043	-2336	-2526	-3253	7622	-2373	-2975	-2839	-3260	-2410	-1722	-2033	-2311	-2848	-663	-860	-2005	-3436	-3250	248
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
243(R)	-870	-2087	-829	1490	-2474	-1760	-229	-2109	1269	-44	-1196	-424	-1829	205	2225	-775	-768	-1753	-2143	-1047	249
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
244(V)	2339	-967	-2970	-2766	-1878	-1847	-2252	32	-2541	-1299	-918	-2087	-2399	-2316	-2545	-1157	-971	2315	-2605	-2251	250
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
245(I)	-1827	-1398	-4307	-3831	-1099	-3930	-3142	3288	-3619	1835	69	-3579	-3671	-3177	-3511	-3178	-1791	1918	-2524	-2310	251
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
246(V)	-1178	-1448	-1943	-1452	-1776	-2261	-1140	-227	1866	-1260	-816	-1444	-2448	-902	-540	-1496	-1176	2694	-2161	-1764	252
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
247(E)	-508	-1976	840	1547	-2280	-1393	-117	-2029	1400	-1984	-1077	1158	-1531	330	-253	-378	-454	262	-2163	-1471	253
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

SUBSTITUTE SHEET (RULE 26)

Table 12

248(M)	1703	-991	-2901	-2342	-528	-2667	-1550	166	-2031	1544	[illegible]	-2104	-2591	-1715	-2010	-1685	-1052	-12	-1442	-1177	254			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
249(I)	-1947	-1516	-4385	-3885	-916	-4013	-3118	2193	-3656	2186	257	-3656	-3687	-3109	-3494	-3250	-1889	1383	-2397	-2258	255			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
250(E)	-1322	-2647	-272	[illegible]	-3071	-1811	-576	-2759	2306	-2633	-1854	-464	-2066	-175	-177	-1144	-1256	-2368	-2692	-2140	256			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
251(K)	-1395	-2058	-1711	-1614	-2215	-2216	-641	-1709	[illegible]	-1652	2576	-1075	-2303	-282	287	-1423	-1293	-1603	-2159	-1803	257			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
252(D)	-1285	-2888	[illegible]	176	-3210	1189	-737	-3047	-715	-2977	-2195	-191	-1979	2196	-1379	-1050	-1315	-2564	-3161	-2320	258			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
253(I)	-2073	-1632	-4434	-3975	-911	-4130	-3230	[illegible]	-3706	1451	244	-3779	-3785	-3187	-3557	-3413	-2021	546	-2449	-2273	259			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
254(K)	-1570	-2144	-1837	-1191	-2093	-2383	-750	-1603	[illegible]	939	-1112	-1231	-2436	-408	215	-1616	-1443	-1580	-2166	-1804	260			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
255(P)	-2931	-2678	-3420	-3708	-4181	-2925	-3468	-4621	-3859	-4490	-4105	-3491	[illegible]	-3761	-3695	-3182	-3279	-4037	-3594	-4064	261			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
256(R)	-928	-1705	-1507	-1055	-2761	-1730	-896	-2490	-44	-2489	-1723	-1042	-2102	-543	[illegible]	2258	-1053	-1998	-2546	-2158	262			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
257(D)	-1280	-2665	[illegible]	176	-3164	1547	-743	-3034	-728	-2071	-2194	-1943	-1079	1342	-1301	553	-1316	-2652	-3161	-2317	263			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
258(I)	-1997	-1562	-4355	-3927	-1042	-4066	-3261	[illegible]	-3654	937	97	-3718	-3783	-3239	-3555	-3364	-1959	702	-2548	-2295	264			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
259(M)	-2252	-1621	-4572	-3991	-530	-4164	-2990	2068	-3709	1993	[illegible]	-3808	-3685	-2916	-3406	-3378	-2149	-172	-2084	-2091	265			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																

Table 12

260(T)	-1213	-1674	-2755	-2606	-3163	-1922	-2659	-2898	-2758	-3105	-2612	-2311	-2600	-2708	-2753	-1463	3619	-2197	-3286	-3156	266
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
261(R)	-2131	-2786	-2704	-1460	-3618	-2638	-587	-2978	1735	-2645	-1985	-1353	-2603	-173	3492	-2020	-1828	-2748	-2484	-2384	267
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
262(K)	-1349	-2635	-381	2083	-3083	-1857	-565	-2750	2890	-2612	-1837	-514	-2090	-161	-61	-1178	-1271	-2369	-2655	-2138	268
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
263(A)	2821	-932	-2451	-2472	-3065	-1196	-2233	-2763	-2434	-3056	-2201	-1633	-1940	-2147	-2468	1831	-730	-1840	-3305	-3055	269
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
264(F)	-2063	-1686	-4037	-3677	3337	-3644	-1706	2063	-3359	135	67	-3095	-3486	-2739	-3127	-2876	-2012	-83	-1038	-158	270
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
265(E)	-2641	-3308	-896	3732	-3966	-2458	-2043	-4105	-2128	-4016	-3555	-1531	-2959	-1842	-2560	-2479	-2750	-3722	-3563	-3385	271
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
266(N)	-1662	-3306	2055	78	-3621	-1643	-1040	-3822	-1272	-3531	-2870	3477	-2182	-724	-2071	-1371	-1757	-3092	-3633	-2700	272
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
267(A)	3438	-1472	-2846	-3040	-3287	-1726	-2736	-2840	-3028	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	273
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
268(I)	-1760	-1307	-4325	-3962	-1735	-4042	-3726	5135	-3828	-579	-515	-3722	-3869	-3673	-3896	-3359	-1752	2276	-3240	-2806	274
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
269(T)	1428	-904	-2334	-2158	-2747	-1206	-1940	-2392	-2037	-2678	-1846	-1504	-1806	-1809	-2163	902	3901	-1635	-2999	-2705	275
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
270(V)	-1745	-1300	-4286	-3858	-1446	-3967	-3370	2358	-3688	852	-261	-3606	-3749	-3403	-3673	-3232	-1717	2643	-2858	-2524	276
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
271(V)	-1404	-1072	-3766	-3305	-1464	-3356	-2696	2276	-3080	-616	-379	-3001	-3325	-2870	-3091	-2563	1344	3321	-2516	-2113	277
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

272(M)	866	-1113	-2656	-2412	-1322	-1920	-1883	-487	-2061	-537	-4361	-1959	-2387	-1928	-2078	-1220	-1053	-498	-2134	-1803	278
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
273(A)	2681	-957	-2898	-2711	-1943	-1740	-2211	-165	-2487	-1406	-1001	-2008	-2320	-2266	-2494	-1053	-929	1990	-2626	-2279	279
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
274(L)	-1171	-983	-3266	-2733	-796	-2795	-1888	590	-2418	2001	198	-2418	-2816	-2106	-2362	-1944	985	1777	-1724	-1426	280
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
275(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	281
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
276(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	282
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
277(S)	-897	-1462	-2333	-2543	-3185	-1640	-2474	-3294	-2656	-3497	-2780	-1973	-2360	-2483	-2703	3465	-1316	-2413	-3310	-3025	283
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
278(T)	-1213	-1674	-2755	-2906	-3163	-1922	-2659	-2898	-2788	-3105	-2612	-2311	-2600	-2708	-2753	-1463	3819	-2197	-3288	-3156	284
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
279(N)	-2171	-2655	-1458	-1748	-3334	-2364	-2267	-3843	-2365	-3936	-3437	4208	-2932	-2205	-2603	-2224	-2439	-3392	-3203	-2909	285
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
280(A)	3134	-934	-2491	-2567	-3083	-1203	-2300	-2766	-2540	-3082	-2237	-1672	-1954	-2240	-2537	874	-747	-1844	-3333	-3093	286
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
281(V)	-984	-1045	-3169	-2909	-1709	-2304	-2404	531	-2643	-988	-697	-2378	-2722	-2480	-2661	-1601	1504	3814	-2589	-2201	287
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
282(L)	-2631	-2159	-4786	-4228	-462	-4506	-3231	96	-3878	3628	2482	-4157	-3680	-3016	-3541	-3793	-2509	-608	-2134	-2182	288
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
283(H)	-3205	-3079	-2723	-2890	-2110	-3046	5295	-4135	-2617	-3813	-3561	-2886	-3482	-2833	-2620	-3291	-3356	-3895	-2397	-1681	289
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

284(L)	-1623	-1338	-3726	-3164	-251	-3255	-1920	1373	-2868	2371	514	-2785	-3086	-2281	-2613	-2389	-1543	-181	-1311	1782	290			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
285(L)	-2333	-1873	-4640	-4127	-650	-4326	-3241	2176	-3843	2819	523	-3962	-3833	-3105	-3579	-3604	-2247	56	-2268	-2230	291			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
286(A)	-1472	-2846	-3040	-3287	-1726	-2735	-2840	-3026	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	292				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
287(M)	-1866	-1507	-4176	-3693	-877	-3806	-2901	3308	-3360	335	3308	-3451	-3570	-2934	-3251	-3944	-1840	524	-2286	-2089	293			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
288(A)	-1472	-2846	-3040	-3287	-1726	-2735	-2840	-3026	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	294				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
289(H)	-1490	-2494	-362	-476	-1616	-1680	4329	-2954	-694	-2770	-2133	2185	-2285	-728	-1000	-1377	-1556	-2475	-2146	-1255	295			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
290(A)	-911	-2326	-2131	-2611	-1197	-1934	-2480	-2011	-2745	-1898	-1496	-1888	-1785	-2153	1898	1075	-1682	-3044	-2749	296				
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
291(I)	2038	-985	-3386	-2919	-1320	-2693	-2277	2166	-2677	-587	-297	-2593	-2992	-2450	-2997	-2087	-1268	1681	-2229	-1840	297			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
292(G)	-1243	-2769	311	1902	-3172	1988	-744	-2992	-697	-2936	-2152	1923	-1974	-377	-1331	-1039	-1284	-2506	-3125	-2308	298			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
293(V)	-1738	-1298	-4281	-3921	-1737	-3979	-3665	1917	-3774	-601	-528	-3671	-3894	-3628	-3843	-3263	-1735	3315	-3215	-2770	299			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	358	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
294(E)	-833	-2344	1092	2412	-2643	-1464	-366	-2413	-146	-2368	-1505	-36	562	29	-717	-666	862	-1966	-2562	-1816	300			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	358	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																
295(W)	-1360	-1116	-3614	-3026	1322	-2981	-1582	1966	-2661	1775	556	-2562	-2865	-2117	-2424	-2098	-1362	-187	2908	-629	301			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378	*																

Table 12

296(T)	-350	-973	-2204	-2178	-2893	-1296	-2035	-2561	-2117	-2862	-2043	-1598	-1946	-1916	-2214	1618	3168	-1758	-3137	-2831	302		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
297(L)	-1443	-1269	-3144	-2576	-528	-3614	-1816	1945	-2155	2162	508	-2422	-2699	1193	-2133	-2129	-1368	-55	-1616	-1384	303		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
298(D)	-1826	-3682	3559	1199	-3883	-1662	-1073	-3846	-1391	-3720	-3110	-272	-2222	-760	-2283	-1471	-1913	-3321	-3864	-2364	304		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
299(D)	-2764	-3432	4018	-1200	-4140	-2466	-2197	-4505	-2621	-4365	-3856	-1551	-3014	-2039	-3232	-2583	-2938	-4046	-3716	-3552	305		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
300(F)	-3342	-2776	-4026	-4232	4284	-3645	-1431	-2315	-4038	-1801	-1900	-3298	-3780	-3356	-3645	-3490	-3426	-2566	-739	-3491	306		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
301(Q)	-1048	-2608	235	2170	-2893	-1535	-505	-2660	-255	-2604	-1769	1814	-1849	2272	-789	-848	-1028	-2228	-2770	-2013	307		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
302(R)	1083	-1687	631	135	-2053	-1406	-478	-1755	214	-1795	-624	-145	-1553	247	1639	-383	1217	-1367	-2051	-1404	308		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
303(I)	-1915	-1536	-4077	-3667	2027	-3676	-2150	5138	-3361	144	94	-3225	-3506	-2848	-3202	-2914	-1871	345	-1522	-791	309		
-	-149	-500	239	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
304(R)	-689	-2015	-494	21	-2395	-1582	-184	-2087	444	-2020	-1151	1161	-1687	1832	2131	626	-614	-1684	-2156	-1573	310		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
305(D)	387	-1967	1608	1358	-2275	-1381	1561	-2025	282	-1976	-1667	-25	-1525	342	1024	-369	-443	-1584	-2152	-1482	311		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
306(R)	-1460	-2315	-1793	-887	-2832	-2237	-431	-2268	2193	-2199	-1473	-946	-2245	-20	2766	-1394	-1275	591	-2248	-1961	312		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															
307(V)	-941	-1027	-3099	-2832	-1692	-2234	-2324	470	-2565	-1003	-695	-2305	-2663	-2399	-2587	-1527	1858	2238	-2536	-2152	313		
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249			
-	-16	-7108	-8150	-894	-1115	-701	-1378	*															

Table 12

308(P)	-2931	-2678	-3420	-3706	-4181	-2925	-3468	-4621	-3859	-4490	-4165	-3491	-4236	-3781	-3695	-3182	-3279	-4087	-3594	-4064	314
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
309(V)	-1090	-1215	-2097	-1824	-819	-2221	2699	-287	-1392	-1027	-591	-1674	-2482	-1446	-1482	-1462	-1143	2879	-1420	-707	315
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
310(L)	-2439	-1972	-4702	-4181	-588	-4401	-3258	1582	-3881	2757	587	-4061	-3862	-3093	-3590	-3689	-2344	-130	-2230	-2217	316
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
311(C)	2157	4166	-3012	-2973	-2780	1022	-2337	-2398	-2724	-2744	-1930	-1766	-1943	-2372	-2623	-540	-662	-1624	-3091	-2861	317
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
312(D)	-1742	-3453	3468	99	-3733	-1645	-1066	-3747	-1356	-3641	-3008	1696	-2201	-755	-2209	-1416	-1833	-3208	-3752	-2776	318
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
313(L)	-2477	-2023	-4713	-4122	1592	-4329	-2920	72	-3835	2593	2472	-3946	-3754	-2914	-3466	-3550	-2350	-634	-1927	-1830	319
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
314(K)	-2620	-2961	-2481	-2046	-3743	-2791	-1570	-3603	3284	-3387	-2839	-2048	-3039	-1260	-465	-2604	-2536	-3331	-3001	-2986	320
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
315(P)	-2931	-2678	-3420	-3706	-4181	-2925	-3468	-4621	-3859	-4490	-4105	-3491	-4236	-3781	-3695	-3182	-3279	-4087	-3594	-4064	321
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
316(S)	-897	-1462	-2333	-2543	-3185	-1640	-2474	-3294	-2686	-3497	-2780	-1973	-2360	-2483	-2703	3468	-1316	-2413	-3310	-3025	322
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
317(G)	-2594	-2690	-3394	-3623	-4328	3772	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	323
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
318(K)	2	-2257	-1073	-374	-2740	-1908	-278	-2339	2328	-2192	-1373	-562	-1953	2273	1344	-952	-933	-1980	-2234	-1799	324
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
319(Y)	-3482	-2868	-3701	-3919	238	-3552	-1112	-3000	-3638	-2516	-2526	-3027	-3772	-3101	-3341	-3418	-3527	-3071	-441	4711	325
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

320(M)	-1559	-1287	-3829	-3380	-1103	-3357	-2655	805	-3067	-84	3048	-3065	-3326	-2779	-3011	-2591	-1556	2855	-2312	-1908	326			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
321(M)	1225	-469	-2256	-1679	1656	-1926	-870	90	-1396	-210	2788	-1424	-2028	-1124	-1411	-1008	712	154	-951	-586	327			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
322(T)	-738	-2094	-84	1704	-2416	-1495	-317	-2135	81	-2127	-1275	-163	-1704	1857	-405	-613	1030	-1734	-2331	-1668	328			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
323(D)	-1746	-3458	3548	90	-3744	-1650	-1081	-3767	-1361	-3652	-3036	1366	-2211	-772	-2239	-1429	-1850	-3226	-3765	-2769	329			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
324(L)	-2451	-1983	-4707	-4186	-582	-4409	-3259	1519	-3654	2776	592	-4069	-3865	-3091	-3590	-3698	-2355	-150	-2226	-2214	330			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
325(H)	-2923	-2573	-2959	-2928	826	-3443	4563	-2508	-2463	-2054	-1948	-2279	-3499	-2191	-2397	-2761	-2855	-2540	123	2920	331			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
326(K)	373	-1957	-342	1025	-2297	-1472	-98	-2618	2133	-1954	-1636	806	-1570	352	885	-424	-473	-1592	-2105	-1469	332			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
327(V)	1739	-1008	-3509	-3043	-1376	-3028	-2400	1765	-2857	-615	-334	-2718	-3085	-2585	-2823	-2226	-1269	2276	-2322	-1931	333			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
328(G)	-2594	-2690	-3304	-3623	-4328	3722	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	334			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
329(G)	-2594	-2690	-3304	-3623	-4328	3722	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	335			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
330(U)	-1756	-1352	-4331	-3970	-1756	-4054	-3748	2979	-3840	-603	-533	-3731	-3877	-3603	-3814	-3372	-1750	2505	-3263	-2824	336			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																
331(P)	-2931	-2878	-3420	-3706	-4181	-2925	-3468	-4621	-3859	-4490	-4165	-3491	4225	-3781	-3695	-3182	-3279	-4087	-3504	-4064	337			
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249				
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*																

Table 12

332(Q)	1795	-1440	-730	-492	-2453	682	-812	-2151	-508	-2256	-1426	-624	-1796	2686	-901	-590	-689	-1636	-2516	-1971	338
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
333(V)	-1771	-1603	-3750	-3689	-2037	-3050	-3231	403	-3479	-1154	-1076	-3246	-3399	-3383	-3437	-2628	-1917	3836	-3074	-2677	339
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
334(M)	-2355	-1988	-4343	-3834	-504	-4051	-2868	105	-3385	1451	4480	-3660	-3671	-2806	-3171	-3327	-2274	-474	-2039	-1925	340
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
335(K)	-2620	-2961	-2461	-2646	-3743	-2791	-1570	-3603	3784	-3387	-2839	-2048	-3039	-1260	-465	-2604	-2536	-3331	-3001	-2968	341
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
336(Y)	-1187	-974	-3186	-2638	-117	-2732	-1255	1905	-2270	73	1977	-2217	-2699	-1882	-2144	-1841	-1124	71	-907	3254	342
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
337(L)	-2871	-2457	-4231	-4103	-1033	-3803	-3165	-541	-3734	3130	-31	-3935	-3797	-3286	-3484	-3713	-2869	-1136	-2394	-2220	343
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
338(L)	-2871	-2457	-4231	-4103	-1033	-3803	-3165	-541	-3734	3130	-31	-3935	-3797	-3286	-3484	-3713	-2869	-1136	-2394	-2220	344
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
339(K)	-864	-1785	-860	-366	-2128	-1763	-407	-1812	2824	-1800	-1045	629	-1900	-28	62	-851	-805	1127	-2064	-1581	345
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
340(N)	602	-1686	-275	1008	-1926	-1415	1528	-1618	244	-1673	-815	1892	-1530	299	-244	-371	-391	322	-1934	-1306	346
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
341(G)	-1709	-2639	1362	-690	-3785	3287	-1671	-3805	-1946	-3792	-3137	-980	-2480	-1424	-2576	-1630	-1936	-3150	-3628	-3156	347
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
342(F)	-942	-799	-2628	-2226	1787	-2476	-1269	1109	581	1793	516	-1952	-2453	-1557	-1815	-1558	-875	52	-1138	-794	348
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
343(L)	-2451	-1983	-4707	-4186	-582	-4409	-3259	1510	-3884	2775	592	-4069	-3865	-3091	-3590	-3698	-2355	-150	-2226	-2214	349
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

344(H)	-3205	-3079	-2723	-2590	-2110	-3046	-5236	-4135	-2617	-3813	-3561	-2856	-3482	-2833	-2620	-3291	-3356	-3895	-2397	-1681	350
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
345(G)	-2594	-2690	-3304	-3623	-4328	-3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	351
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
346(D)	-2784	-3432	-4018	-1200	-4140	-2466	-2197	-4505	-2621	-4365	-3956	-1551	-3014	-2039	-3232	-2593	-2938	-4046	-3710	-3552	352
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
347(C)	774	-4459	-2162	-1688	-1962	-1478	-1302	-1474	-944	-1796	-1086	-1351	-1979	-1147	1684	-732	-719	-1116	-2235	-1861	353
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
348(L)	-2387	-1922	-4674	-4155	-617	-4366	-3250	1889	-3865	2650	558	-4023	-3847	-3098	-3586	-3647	-2296	-38	-2247	-2224	354
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
349(T)	-1213	-1674	-2755	-2906	-3163	-1922	-2659	-2698	-2788	-3105	-2612	-2311	-2600	-2708	-2753	-1463	3819	-2197	-3286	-3156	355
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
350(C)	-1489	3972	-4007	-3563	-1524	-3541	-2939	2812	-3350	-617	-413	-3224	-3470	-3129	-3335	-2770	-1475	2269	-2657	-2246	356
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
351(T)	-364	-979	-2232	-2250	-2904	-1245	-2090	-2559	-2191	-2881	-2075	-1071	-1904	-1991	-2260	905	3428	-1762	-3109	-2858	357
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
352(G)	-2594	-2690	-3304	-3623	-4328	-3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	358
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
353(K)	-1716	-2632	-2004	-1008	-3836	-2370	-444	-2764	-2775	-2484	-1756	-1035	-2357	2151	1811	-1592	-1477	-2481	-2391	-2172	359
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
354(T)	-1213	-1674	-2755	-2906	-3163	-1922	-2659	-2698	-2788	-3105	-2612	-2311	-2600	-2708	-2753	-1463	3819	-2197	-3286	-3156	360
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
355(V)	-1771	-1339	-4275	-3816	-1235	-3919	-3194	2139	-3617	1520	-66	-3558	-3681	-3244	-3547	-3164	-1733	3390	-2634	-2369	361
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

356(A)	8433	-1472	-2846	-3040	-3287	-1726	-2735	-2840	-3028	-3257	-2862	-2238	-2447	-2798	-2944	-1216	-1357	-2183	-3405	-3320	362
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
357(E)	-2641	-3308	-896	3292	-3966	-2458	-2043	-4105	-2128	-4016	-3555	-1531	-2959	-1842	-2560	-2479	-2750	-3722	-3563	-3385	363
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
358(N)	-823	-1917	-96	1188	-2187	-1547	-506	-1711	-265	-1955	-1191	2711	-1815	-144	-747	-757	-815	1140	-2297	-1666	364
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
359(L)	-2153	-1779	-4360	-3884	-675	-3965	-3012	392	-3561	9728	467	-3673	-3662	-2955	-3355	-3239	-2102	1281	-2207	-2099	365
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
360(E)	1136	-2084	-175	2627	-2436	-1610	-274	-2147	1525	-2118	-1254	-175	-1692	152	-251	-593	-670	-1736	-2296	-1650	366
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
361(H)	893	-1761	1357	214	-2092	-1387	1862	-1810	229	-1825	-942	-83	-1527	293	-273	640	793	-1409	-2050	-1397	367
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
362(I)	608	-458	-2776	-2176	1666	-2202	-1113	1712	-1836	-222	338	-1762	-2245	-1512	-1731	-1292	867	1366	-1038	-884	368
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
363(P)	-922	-1912	1681	-141	-2123	-1604	-687	-1787	-550	187	-1245	-427	2037	-369	-1049	-882	-947	-1524	-2338	-1711	369
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
364(D)	-1692	-3605	3364	1256	-3770	-1599	-957	-3709	-1216	-3589	-2909	1025	-2138	-628	-2083	-1346	-1761	-3174	-3765	-2738	370
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
365(Q)	-877	-1646	-633	499	-1610	-1781	-505	-1210	-63	1648	-649	-558	-1031	2241	-360	-907	-814	-1097	-1882	-1385	371
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
366(P)	-648	-2019	1139	203	-2354	-1436	-265	-2089	29	-2086	-1217	-114	1966	1445	-492	-529	1244	-1672	-2300	-1616	372
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-571	-7108	-1646	-894	-1115	-701	-1378	*	*												
367(R)	-422	-1089	-851	-304	1406	-1496	-183	-740	147	-894	-230	-440	775	21	2009	-539	-381	-568	-1136	-521	373
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-23	-8580	-7602	-894	-1115	-341	-2249	*	*												

Table 12

368(D)	1472	-1668	1838	-70	-2356	-1385	-511	-2062	-246	-2128	-1275	-318	1359	-418	-746	-526	425	-1602	-2386	-1752	374
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
369(G)	-1044	-2230	2141	-100	-3222	2291	-982	-3045	-1033	-3050	-2258	-395	-1985	-644	-1669	853	-1207	-2428	-3250	-2493	375
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
370(Q)	-2562	-2904	-1886	-1971	-3251	-2661	-2079	-3690	-1565	-3469	-3081	-2107	-3091	4371	-1665	-2585	-2674	-3411	-3077	-2821	376
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
371(D)	-1275	-2955	2862	1330	-3205	-1556	-670	-3029	1509	-2936	-2141	-158	-1955	-290	-1213	-1025	-1261	-2554	-3111	-2272	377
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
372(V)	-1738	-1298	-4281	-3921	-1737	-3979	-3665	1917	-3774	-601	-528	-3671	-3834	-3628	-3843	-3293	-1735	3205	-3215	-2770	378
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-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
373(I)	-2091	-1746	-3971	-3840	-1676	-3532	-3289	3664	-3581	-659	-693	-3562	-3674	-3445	-3521	-3194	-2146	449	-2877	-2493	379
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-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
374(M)	-584	-1354	-847	-248	-1467	-1659	2505	-1087	212	-374	2671	-449	-1729	1171	1074	-634	-507	-876	-1617	-1126	380
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
375(P)	-910	-2031	-79	1195	-2792	-1486	-794	-2539	-629	-2588	-1788	-401	3061	-439	-1131	812	-1014	-2050	-2815	-2151	381
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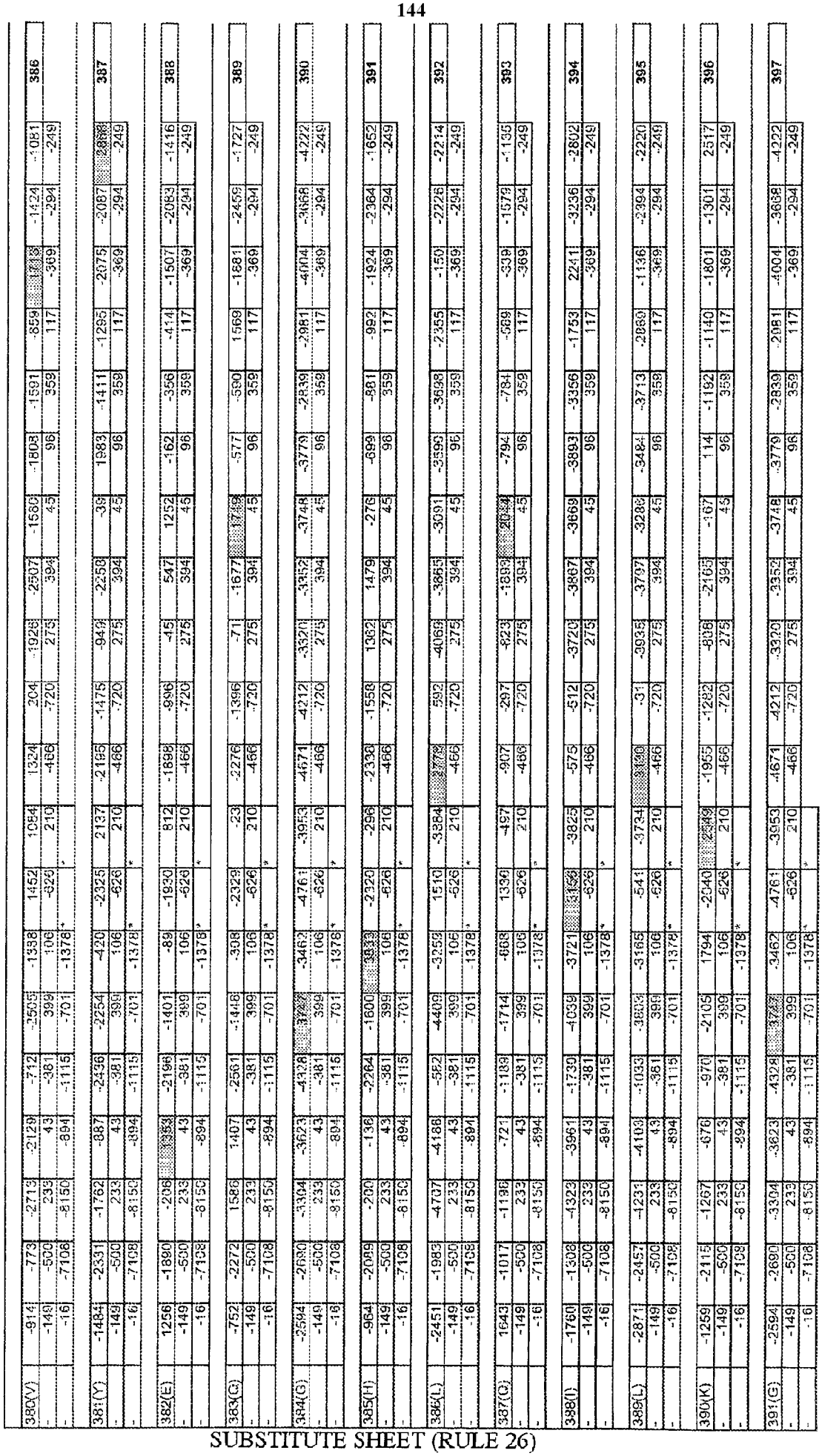
376(W)	-1588	-1300	-3783	-3197	-329	-3245	-1926	2071	-2827	1901	558	-2822	-3072	-2297	-2616	-2381	-1508	-111	3483	-1042	382
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
377(E)	-1024	-2640	1844	2239	-2968	-1498	-505	-2711	-344	-2636	-1791	-107	-1824	1521	-957	207	-1011	-2243	-2817	-2021	383
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
378(N)	-826	-2349	1089	227	-2651	-1487	-341	-2416	1494	-2346	-1475	2601	-1724	1006	-522	-657	-787	-1968	-2511	-1791	384
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
379(P)	1932	-1116	-2232	-2301	-3058	-1358	-2206	-2706	-2336	-3009	-2238	-1674	3274	-2114	-2406	-739	-914	-1913	-3260	-3019	385
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12



380(V)	-914	-773	-2719	-2129	-712	-2505	-1338	1452	1054	1324	304	-1928	-2507	-1580	-1803	-1591	-859	118	-1424	-1081	386
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-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
381(Y)	-1484	-2331	-1762	-887	-2436	-2254	-420	-2325	2137	-2165	-1475	-949	-2258	-39	1983	-1411	-1295	-2075	-2087	-2888	387
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-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
382(E)	1256	-1890	-206	1353	-2196	-1401	-89	-1930	812	-1898	-996	-45	547	1252	-162	-356	-414	-1507	-2083	-1416	388
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
383(G)	-752	-2272	1586	1407	-2561	-1446	-308	-2329	-23	-2276	-1396	-71	-1677	1748	-577	-590	1569	-1681	-2459	-1727	389
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
384(G)	-2594	-2680	-3304	-3623	-4328	3773	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	390
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-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
385(H)	-964	-2089	-200	-136	-2264	-1600	3833	-2320	-296	-2330	-1558	1362	1479	-276	-699	-861	-992	-1924	-2364	-1652	391
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
386(L)	-2451	-1983	-4707	-4186	-562	-4409	-3259	1510	-3384	3773	592	-4069	-3865	-3091	-3595	-3638	-2355	-150	-2226	-2214	392
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
387(Q)	1643	-1017	-1196	-721	-1188	-1714	-668	1336	-497	-907	-297	-823	-1883	2044	-794	-784	-569	-339	-1579	-1135	393
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-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
388(I)	-1760	-1308	-4323	-3961	-1730	-4039	-3721	3158	-3825	-575	-512	-3720	-3867	-3669	-3893	-3356	-1753	2241	-3236	-2802	394
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-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
389(L)	-2871	-2457	-4231	-4103	-1033	-3833	-3165	-541	-3734	3139	-31	-3935	-3797	-3286	-3484	-3713	-2069	-1136	-2994	-2220	395
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-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
390(K)	-1259	-2115	-1267	-676	-970	-2105	1794	-2540	2549	-1955	-1282	-838	-2165	-167	114	-1182	-1140	-1801	-1301	2517	396
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-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	
391(G)	-2594	-2680	-3304	-3623	-4328	3773	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	397
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*	*	*	*	*	*	*	*	*	*	*	*	*	*	

Table 12

392(N)	-2171	-2655	-1458	-1748	-3334	-2364	-2267	-3943	-3365	-3936	-3437	-3008	-2932	-2205	-2603	-2224	-2439	-3392	-3253	-2903	398
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
393(L)	-2871	-2457	-4231	-4108	-1033	-3603	-3165	-541	-3734	-3130	-31	-3935	-3797	-3283	-3484	-3713	-2669	-1136	-2394	-2220	399
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
394(A)	-3331	-934	-2489	-2561	-3081	-1203	-2295	-2766	-2533	-3080	-2234	-1669	-1953	-2234	-2533	936	-746	-1844	-3331	-3060	400
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
395(E)	-522	-1773	-240	-3688	-2248	-1396	-238	-1960	50	-1989	-1115	-174	1198	131	-443	1226	677	-1538	-2214	-1565	401
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
396(E)	-1481	-3230	1425	-3686	-3481	751	-843	-3354	-954	-3256	-2520	-187	-2657	-492	-1711	-1193	-1527	-2812	-3445	-2523	402
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-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
397(G)	-2594	-2690	-3304	-3623	-4328	3743	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	403
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
398(A)	-2843	-932	-2454	-2477	-3068	-1138	-2036	-2763	-2439	-3057	-2202	-1635	-1940	-2152	-2471	1777	-731	-1843	-3308	-3056	404
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
399(V)	-1771	-1623	-3756	-3688	-2037	-3050	-3231	403	-3479	-1154	-1076	-3246	-3398	-3363	-3437	-2628	-1917	-3338	-3074	-2677	405
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
400(A)	-3338	-1472	-2846	-3040	-3287	-1726	-2735	-2940	-3028	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	406
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
401(K)	-2620	-2961	-2461	-2046	-3743	-2791	-1570	-3603	-2838	-3387	-2839	-2048	-3099	-1266	-465	-2604	-2536	-3331	-3001	-2988	407
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
402(I)	-1761	-1312	-4317	-3954	-1713	-4027	-3703	3223	-3814	-556	-498	-3712	-3858	-3633	-3877	-3341	-1754	2110	-3216	-2787	408
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
403(S)	-348	-981	-2200	-2194	-2989	-1227	-2073	-2686	-2157	-2970	-2136	-1541	-1946	-1946	-2253	2353	1398	-1824	-3217	-2916	409
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
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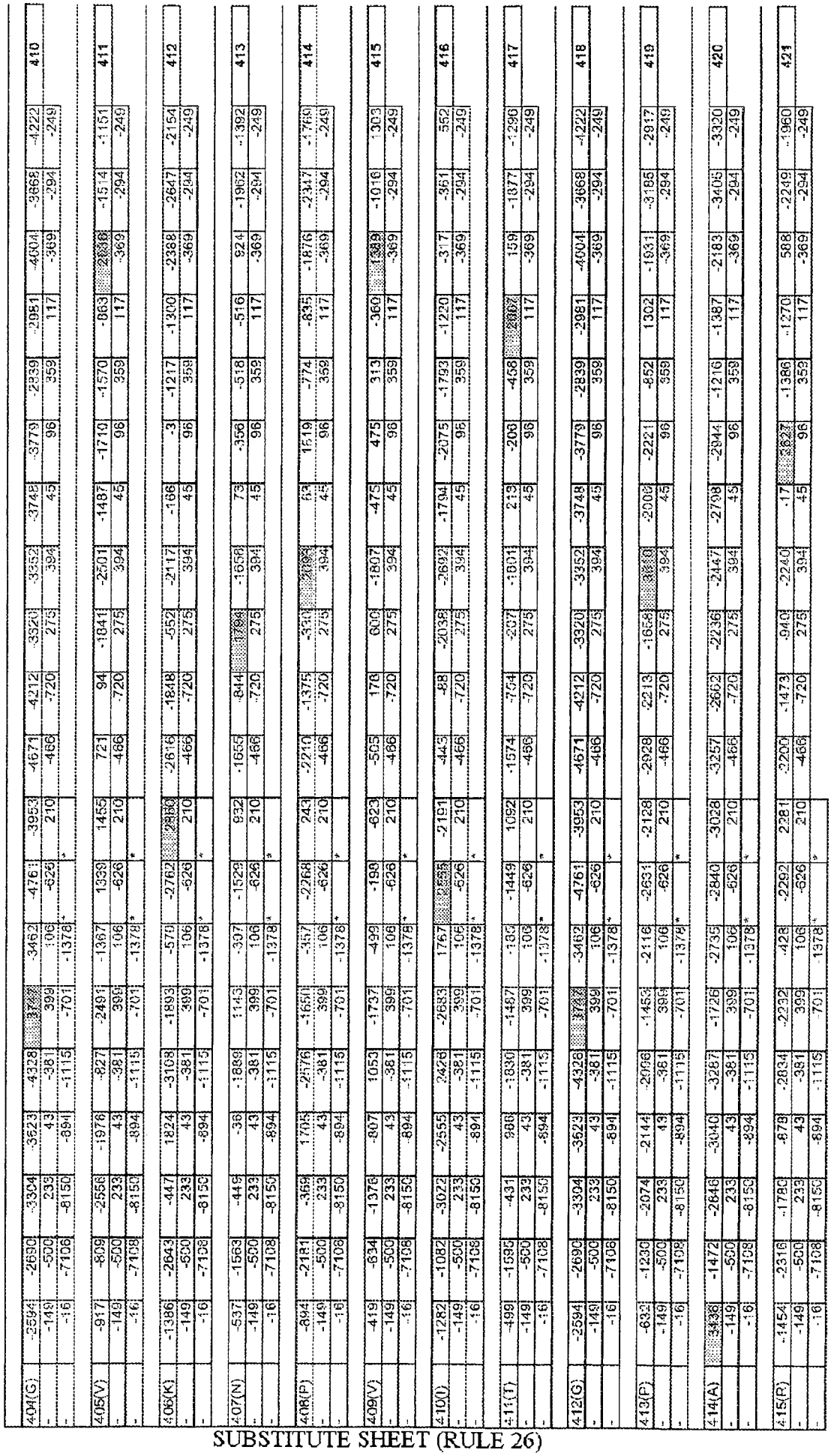
Table 12



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-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*														
405(V)	-917	-809	-2556	-1976	-827	-2491	-1367	1338	1455	721	94	-1841	-2501	-1487	-1710	-1570	-863	[illegible]	-1514	-1151	411
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*														
406(K)	-1386	-2643	-447	1824	-3108	-1893	-570	-2762	[illegible]	-2616	-1848	-552	-2117	-166	-3	-1217	-1300	-2388	-2647	-2154	412
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*														
407(N)	-537	-1563	-446	-36	-1889	1143	-307	-1529	932	-1655	-844	[illegible]	-1656	73	-356	-518	-516	924	-1962	-1392	413
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*														
408(P)	-894	-2181	-369	1705	-2576	-1650	-357	-2268	243	-2210	-1375	-391	[illegible]	63	1619	-774	-835	-1876	-2347	-1760	414
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*														
409(V)	-419	-634	-1376	-807	1053	-1737	-499	-199	-623	-505	176	600	-1807	-475	475	313	-360	[illegible]	-1016	1303	415
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*														
410(I)	-1282	-1082	-3022	-2555	2426	-2683	1767	[illegible]	-2191	-445	-88	-2038	-2692	-1794	-2075	-1793	-1220	-317	-361	552	416
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-891	-1115	-701	-1378*														
411(T)	-499	-1696	-431	986	-1830	-1467	-185	-1449	1092	-1574	-754	-207	-1801	219	-208	-458	[illegible]	159	-1877	-1296	417
-	-149	-500	239	43	-381	399	106	-626	210	-456	-720	278	394	45	98	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*														
412(G)	-2594	-2690	-3304	-3623	-4326	[illegible]	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4604	-3668	-4222	418
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	98	358	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378*														
413(F)	-632	-1230	-2074	-2144	2966	-1455	-2116	-2631	-2128	-2928	-2213	-1654	[illegible]	-2005	-2221	-852	1302	-1931	-3185	-2917	419
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-	-16	-7108	-8150	-894	-1115	-701	-1378*														
414(A)	[illegible]	-1472	-2646	-3040	-3287	-1726	-2735	-2840	-3028	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	420
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-	-16	-7108	-8150	-894	-1115	-701	-1378*														
415(R)	-1454	-2316	-1780	-878	-2834	-2232	-428	-2292	2281	-3200	-1473	-940	-2240	-17	[illegible]	-1386	-1270	588	-2249	-1960	421
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-	-16	-7108	-8150	-894	-1115	-701	-1378*														

Table 12

416(V)	-1771	-1603	-3750	-3689	-2037	-3050	-3231	403	-3479	-1154	-1076	-3246	-3399	-3383	-3437	-2828	-1917	3838	-3074	-2677	422
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
417(F)	-3342	-2776	-4026	-4232	4364	-3545	-1431	-2315	-4038	-1801	-1900	-3299	-3780	-3350	-3645	-3490	-3420	-2566	-759	349	423
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
418(D)	-1572	-3426	2573	2447	-3613	-1583	-879	-3513	-1050	-3393	-2684	1292	-2085	-536	-1855	-1253	-1623	-3000	-3585	-2609	424
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
419(S)	-879	-1989	1496	-177	-3045	1600	-939	-2843	-904	-2867	-2046	-438	-1922	-591	-1483	2171	-1044	-2226	-3072	-2372	425
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
420(E)	-2641	-3308	-896	3737	-3966	-2458	-2043	-4105	-2128	-4016	-3555	-1531	-2959	-1842	-2560	-2479	-2750	-3722	-3563	-3385	426
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
421(Q)	-705	-1925	-199	2112	917	-1534	-288	-1824	42	-1842	-1054	-210	-1709	2163	-420	-611	-656	-1502	-1997	-1291	427
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
422(H)	-569	-2048	1450	1526	-2349	-1405	1830	-2103	181	-2058	-1157	-37	-1569	272	-349	713	620	-1662	-2240	-1537	428
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
423(C)	1626	2678	-2671	-2107	1264	-1968	-1091	233	-1777	-334	260	-1072	-2128	-1459	-1691	-1086	-529	1209	-1066	-704	429
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
424(M)	-2042	-1634	-4379	-3826	-659	-3976	-2899	2765	-3546	1204	3685	-3605	-3604	-2896	-3318	-3183	-1961	195	-2135	-2058	430
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
425(E)	412	-2447	1356	2979	-2747	-1477	-445	-2527	-243	-2477	-1622	-107	855	-38	-831	-730	-894	-2073	-2668	-1906	431
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
426(A)	2822	-1031	-2418	-2539	-3226	1898	-2364	-2941	-2626	-3229	-2379	-1722	-2026	-2302	-2634	-654	-848	-1983	-3415	-3226	432
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
427(I)	-1772	-1325	-4307	-3677	-1405	-3993	-3383	2935	-3705	820	-217	-3632	-3761	-3406	-3682	-3260	-1742	2033	-2838	-2525	433
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											

Table 12

428(L)	-875	-1634	-575	959	-1581	-1769	-525	-1179	-135	1884	-625	-547	-1931	1405	-450	-909	-816	-1674	-1883	-1383	434
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
429(A)	1706	-1826	-180	949	-2318	-1410	-359	-2041	-53	-2067	-1204	1001	-1652	52	-561	1232	-595	-1609	-2298	-1643	435
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
430(D)	-1074	-2458	2381	60	-2921	1927	-658	-2710	-463	-2675	-1860	-271	-1918	-276	866	-915	-1100	-2245	-2845	-2124	436
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
431(K)	-668	-2117	785	888	-2469	-1529	-187	-2189	2380	-2106	-1221	-162	-1661	256	1134	-553	-619	-1760	-2240	-1607	437
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
432(I)	-2019	-1582	-4380	-3941	-1000	-4086	-3253	3295	-3671	1100	145	-3736	-3783	-3222	-3556	-3378	-1976	657	-2517	-2289	438
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
433(Q)	-480	-1797	-369	171	-2078	-1457	1762	-1779	1157	-1780	-905	1165	-1550	1798	-48	-396	-422	725	-1966	-1366	439
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
434(A)	1854	-1836	1733	-180	-2714	-1429	-806	-2438	-879	-2518	-1698	-430	1775	-448	-1211	-736	-894	-1923	-2765	-2117	440
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
435(G)	-2594	-2690	-3304	-3623	-4328	3787	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	441
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
436(D)	-1736	-3455	3490	97	-3737	-1646	-1070	-3753	-1363	-3647	-3016	1602	-2204	-760	-2218	-1420	-1838	-3213	-3756	-2780	442
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
437(V)	-1721	-1302	-4229	-3874	-1705	-3894	-3582	1607	-3706	-582	-513	-3610	-3786	-3559	-3767	-3209	-1725	3394	-3158	-2712	443
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
438(V)	594	-988	-3391	-2911	-1164	-2888	-2167	845	-2637	765	-154	-2576	-2962	-2387	-2622	-2074	-1205	2800	-2084	-1724	444
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
439(V)	-1771	-1603	-3750	-3689	-2037	-3050	-3231	403	-3479	-1154	-1076	-3246	-3399	-3383	-3437	-2628	-1917	3508	-3074	-2677	445
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											

Table 12

440(I)	-1754	-1308	-4295	-3567	-1434	-3978	-3377	286	-3697	862	247	-3617	-3754	-3406	-3579	-3243	-1725	2373	-2852	-2526	446
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
441(R)	-2957	-3022	-3318	-2735	-3796	-2998	-1968	-3912	-846	-3631	-3157	-2611	-3280	-1724	4038	-3026	-2913	-3650	-3096	-3185	447
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
442(Y)	-1321	-1438	-1994	-1608	2186	527	-450	-1117	-1481	-1211	-693	1178	-2522	-1217	-1665	-1518	-1275	-1021	-198	3178	448
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
443(C)	-675	2256	-2544	972	-572	-2236	-1121	1373	-1671	679	261	-1700	-2270	-1403	-1668	-1311	-621	1601	-1150	-790	449
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
444(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	450
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
445(P)	-2931	-2878	-3420	-3706	-4181	-2925	-3468	-4621	-3859	-4490	-4165	-3491	4325	-3781	-3695	-3182	-3279	-4087	-3594	-4064	451
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
446(K)	-1060	-2058	-1088	-460	-2452	-1917	-357	-1970	2801	-1978	-1220	-832	-1990	1339	367	-999	-946	536	-2145	-1717	452
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
447(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	453
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
448(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	454
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
449(P)	-2931	-2878	-3420	-3706	-4181	-2925	-3468	-4621	-3859	-4490	-4165	-3491	4325	-3781	-3695	-3182	-3279	-4087	-3594	-4064	455
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
450(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	456
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
451(M)	-2406	-2296	-3638	-3594	-1525	-3105	-2824	-1047	-3121	-596	5043	-3293	-3425	-3046	-2996	-2911	-2552	-1398	-2513	-2207	457
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

452(P)	-1659	-2241	-2022	-1646	-3185	-2242	-1373	-3000	-450	-2936	-2274	-1624	3436	-1065	2095	-1730	-1750	-2593	-2816	-2613	458
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
453(E)	-2641	-3308	-696	2752	-3966	-2458	-2043	-4105	-2128	-4016	-3555	-1531	-2959	-1842	-2560	-2479	-2750	-3722	-3563	-3385	459
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
454(M)	-2406	-2296	-3638	-3594	-1525	-3105	-2824	-1047	-3121	-596	5018	-3293	-3425	-3046	-2996	-2911	-2552	-1398	-2513	-2207	460
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
455(L)	-2871	-2457	-4231	-4103	-1033	-3803	-3165	-541	-3734	3130	-31	-3935	-3797	-3286	-3484	-3713	-2869	-1136	-2394	-2220	461
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
456(K)	1368	-1481	-763	-332	-2319	-1417	-551	-1898	1786	-2068	-1221	-500	-1721	-168	-470	1631	-587	-1532	-2299	-1754	462
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
457(P)	-1500	-1738	-2514	-2380	-1555	-2358	-2022	-1126	-2063	1224	-641	-2189	3436	-2061	-2129	-1822	-1674	-1231	-2290	-1878	463
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
458(T)	-351	-974	-2208	-2185	-2894	-1237	-2041	-2561	-2125	-2863	-2646	-1539	-1948	-1923	-2218	1543	3230	-1758	-3139	-2834	464
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
459(S)	-897	-1462	-2339	-2943	-3186	-1640	-2474	-3294	-2686	-3497	-2780	-1973	-2360	-2463	-2703	2408	-1316	-2413	-3310	-3025	465
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
460(M)	2706	-986	-2433	-2144	-1502	-1684	-1706	-700	-1858	-968	2744	-1705	-2188	-1713	-1932	-963	-862	-592	-2145	-1794	466
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
461(I)	-2103	-1659	-4461	-3902	-869	-4152	-3233	3982	-3723	1619	290	-3801	-3788	-3171	-3557	-3432	-2046	487	-2418	-2266	467
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
462(I)	-1761	-1312	-4317	-3954	-1713	-4027	-3703	3225	-3814	-556	-498	-3712	-3859	-3653	-3877	-3344	-1754	2110	-3216	-2787	468
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
463(C)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	469
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

464(K)	1641	-2033	-323	914	-2415	-1565	-296	-2097	2052	-2080	-1233	-257	-1736	125	-133	-846	-702	-1707	-2258	-1657	470
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
465(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	471
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
466(L)	-1699	-1807	-2268	-1925	-830	-2795	-1551	-455	-1225	2516	90	-1958	-2845	1927	-1308	-2067	-1651	-846	-1841	-1454	472
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
467(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	473
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
468(D)	-853	-2415	2115	1717	-2702	-1468	-378	-2484	1085	-2417	-1546	-84	-1732	41	-599	696	-824	-2025	-2594	-1839	474
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
469(S)	-892	-1780	-931	-688	-2757	-1643	-830	-2472	1671	-2492	-1708	-799	-2010	-468	-365	2676	-1004	-1981	-2598	-2130	475
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
470(C)	-1135	3503	-3700	-3408	-1670	-2549	-2675	853	-3101	-916	-667	-2727	-2925	-2870	-3030	-1868	-1288	2927	-2619	-2222	476
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
471(A)	2690	-1035	-2404	-2530	-3236	2290	-2365	-2804	-2627	-3240	-2389	-1719	-2027	-2302	-2637	-656	-851	-1991	-3423	-3234	477
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
472(L)	-2632	-2152	-4630	-4185	1767	-4324	-2442	-61	-3879	2789	563	-3833	-3823	-2970	-3513	-3609	-2518	-738	-1527	-945	478
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
473(I)	-2073	-1632	-4434	-3975	-911	-4130	-3238	3164	-3706	1451	244	-3779	-3785	-3187	-3557	-3413	-2021	546	-2449	-2273	479
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
474(T)	-1213	-1674	-2755	-2906	-3163	-1922	-2658	-2698	-2788	-3105	-2612	-2311	-2600	-2708	-2753	-1463	3819	-2197	-3286	-3156	480
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
475(D)	-2784	-3432	4016	-1200	-4140	-2466	-2197	-4505	-2621	-4365	-3956	-1551	-3014	-2039	-3232	-2593	-2938	-4046	-3710	-3552	481
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

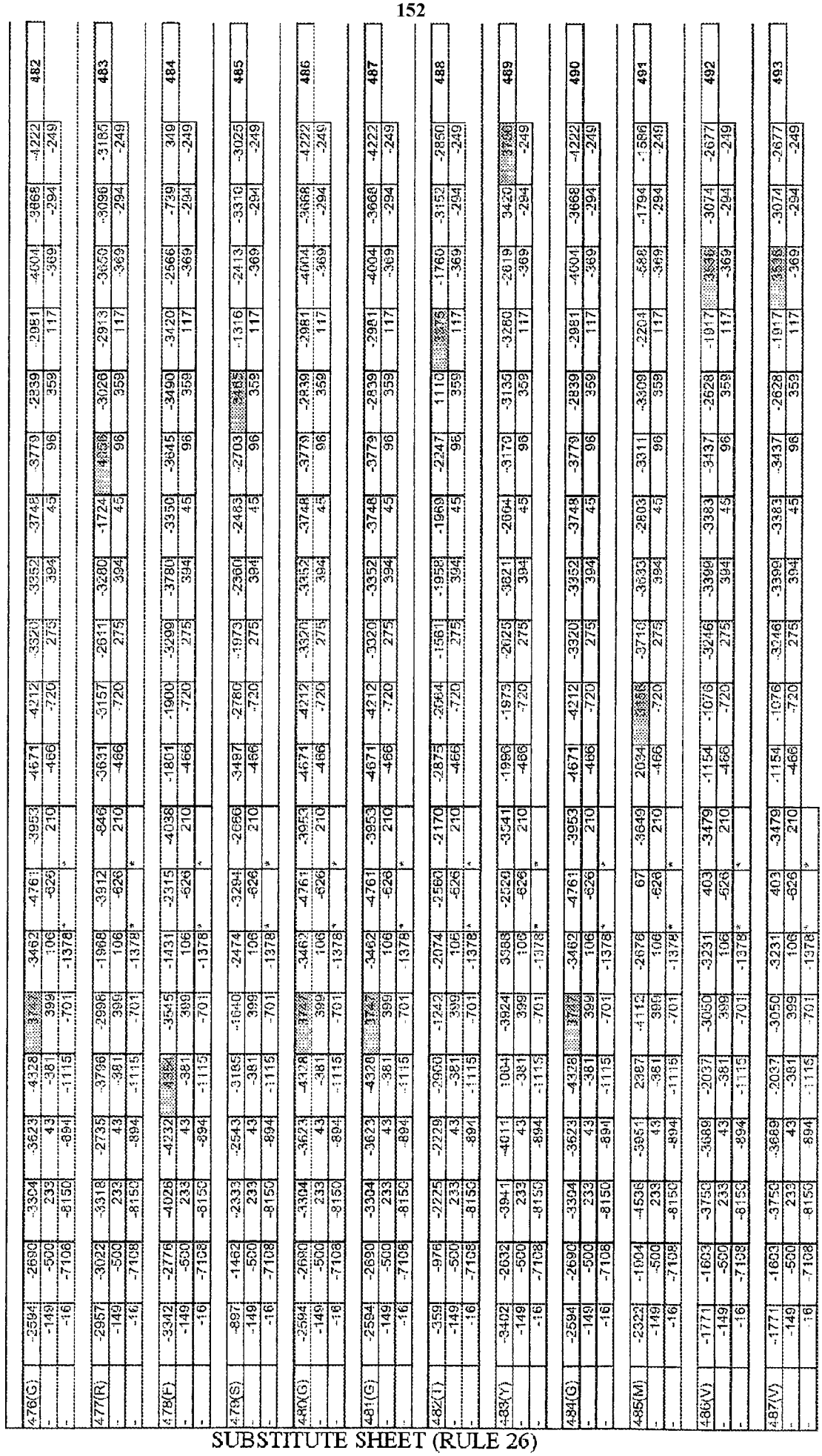


Table 12

488(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	494
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
489(H)	-3205	-3079	-2723	-2890	-2110	-3046	5235	-4135	-2617	-3813	-3561	-2886	-3482	-2833	-2620	-3291	-3356	-3895	-2397	-1681	495
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
490(V)	-1754	-1297	-4329	-3968	-1770	-4053	-3752	2604	-3840	-621	-545	-3728	-3878	-3699	-3917	-3370	-1746	2859	-3276	-2829	496
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
491(A)	2567	-828	-2477	-2155	-1637	-1466	-1728	-743	-1941	-1564	-854	-1607	-2033	-1725	-2034	-738	1178	1108	-2316	-1972	497
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
492(P)	-2931	-2878	-3420	-3706	-4181	-2925	-3468	-4621	-3859	-4490	-4165	-3491	4239	-3781	-3695	-3182	-3279	-4087	-3594	-4064	498
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
493(E)	-2641	-3308	-896	3732	-3966	-2458	-2043	-4105	-2128	-4016	-3555	-1531	-2959	-1842	-2560	-2479	-2750	-3722	-3563	-3385	499
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
494(A)	3436	-1472	-2846	-3040	-3287	-1726	-2735	-2840	-3028	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	500
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
495(Y)	-866	-976	-1869	-1931	1353	-2145	1318	-556	-1116	-777	-173	-1242	-2197	1714	-1301	-1173	-802	888	-445	2739	501
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
496(D)	417	-1831	1617	1094	-2065	-1488	-953	-1618	-107	-1820	-1019	-189	-1698	30	-623	-603	-843	1629	-2154	-1520	502
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
497(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	503
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
498(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	504
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
499(T)	492	-1190	-708	-181	-1475	311	-333	-1099	-81	71	-509	570	1113	-6	-509	-450	1123	-835	-1680	-1161	505
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

500(I)	-2081	-1746	-3971	-3640	-1676	-3532	-3239	3884	-3561	-659	693	-3562	-3674	-3445	-3521	-3194	-2146	449	-2877	-2493	506
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
501(A)	3103	-1036	-2445	-2572	-3222	1051	-2380	-2930	-2650	-3226	-2381	-1739	-2034	-2327	-2648	-664	-857	-1981	-3412	-3226	507
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
502(L)	-2239	-1892	-3711	-3400	301	-3520	-1210	-542	-2948	2564	-35	-2786	-3395	-2438	-2750	-2747	-2185	-945	-573	2562	508
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
503(V)	-1757	-1387	-4101	-3681	-1174	-3714	-3031	880	-3410	1254	-60	-3407	-3585	-3094	-3354	-2984	-1743	3618	-2536	-2219	509
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
504(Q)	-982	-2251	-866	971	-2711	-1822	-252	-2340	1444	-2194	-1356	-464	-1885	2646	1632	-858	-863	-1958	-2245	-1765	510
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
505(E)	-1162	-2771	2137	2233	-3046	-1526	-626	-2849	-546	-2792	-1983	-145	-1905	-242	-1192	-940	1396	-2385	-2990	-2169	511
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
506(G)	-1707	-2684	1591	-814	-3783	3100	-1613	-3795	-1887	-3775	-3119	-915	-2456	-1358	-2539	-1610	-1924	-3150	-3656	-3124	512
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
507(D)	-2784	-3432	4818	-1200	-4140	-2466	-2197	-4505	-2621	-4360	-3856	-1051	-3014	-2039	-3232	-2583	-2936	-4046	-3710	-3552	513
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
508(M)	-473	-522	-1819	-1236	-468	-1879	-687	1519	-996	586	1677	-1154	-1937	836	-1131	1079	-413	102	-957	-585	514
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
509(I)	-1761	-1312	-4317	-3954	-1713	-4027	-3703	3226	-3814	-556	-498	-3712	-3859	-3653	-3877	-3344	-1754	2110	-3216	-2787	515
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
510(T)	782	-1467	-550	1029	-2202	-1425	-709	-1791	-472	-1993	-1203	-526	-1787	-368	-902	-617	2685	-1400	-2333	-1783	516
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
511(I)	-1766	-1333	-4283	-3923	-1635	-3967	-3619	3388	-3759	-473	-437	-3672	-3822	-3576	-3804	-3285	-1764	1695	-3126	-2717	517
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

512(D)	-2784	-3432	-1016	-1200	-4140	-2486	-2197	-4505	-2621	-4365	-3956	-1551	-3014	-2038	-3232	-2593	-2938	-4046	-3710	-3552	518
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
513(A)	-2706	-1451	-1036	-913	-2506	-1504	-1143	-2174	-794	-2337	-1613	-946	-1993	2040	-1061	-809	-910	-1703	-2633	-2156	519
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
514(H)	-615	-1680	1444	66	-1883	168	2650	-1558	-86	-1691	-891	-223	-1680	31	-577	-571	-585	1267	-2007	-1397	520
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
515(K)	-654	-2036	-546	42	-2376	-1581	-133	-2066	1833	-1987	-1107	1132	-1658	1043	1058	-540	1160	-1660	-2113	-1532	521
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
516(N)	-933	-2085	-946	-284	-2472	-1822	-253	-2090	1711	76	-1204	1938	-1876	175	1799	-841	-817	-1755	-2132	-1663	522
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
517(E)	-416	-987	-843	1107	-1070	-1583	-338	-623	-183	879	-172	-489	-1679	-94	-565	544	813	265	-1379	-905	523
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
518(I)	-2258	-1804	-4588	-4084	-708	-4269	-3231	2627	-3807	2252	485	-3923	-3814	-3118	-3570	-3544	-2181	199	-2303	-2237	524
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
519(G)	-477	-1909	958	282	-2211	-1389	1484	-1853	285	-1921	-1016	-32	-1517	2318	-225	830	559	-1525	-2110	-1430	525
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
520(L)	-2127	-1743	-4402	-3796	1257	-3918	-2674	149	-3492	2527	2164	-3553	-3509	-2714	-3181	-3095	-2019	570	-1870	-1818	526
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
521(N)	-723	-2217	958	236	-2518	-1466	1611	-2279	1719	-2217	-1334	2265	-1666	166	-401	-570	-677	-1837	-2382	-1678	527
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
522(V)	-1754	-1297	-4330	-3968	-1770	-4053	-3752	2623	-3841	-620	-545	-3729	-3878	-3699	-3818	-3371	-1746	2846	-3277	-2830	528
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
523(S)	1545	-974	-2003	-1825	-2867	-1206	-1790	-2580	-1788	-2795	-1932	-1362	1826	-1586	-1999	2362	-672	-1755	-3057	-2721	529
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12

524(D)	-1776	-3649	3328	1869	-3838	-1642	-1031	-3788	-1322	-3660	-3029	-245	-2192	-711	-2201	-1425	-1855	-3264	-3821	-2816	530
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
525(E)	423	-2950	1944	2896	-3223	-1545	-718	-3047	-715	-2979	-2196	-161	-1968	-347	-1403	-1043	-1314	-2569	-3177	-2316	531
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
526(E)	-2641	-3308	-896	2732	-3966	-2458	-2043	-4105	-2128	-4016	-3655	-1531	-2959	-1842	-2560	-2479	-2750	-3722	-3563	-3385	532
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
527(L)	-2339	-1699	-4616	-4042	1570	-4204	-2648	1440	-3758	2556	676	-3625	-3700	-2902	-3418	-3418	-2226	-382	-1924	-1778	533
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
528(A)	2338	-1980	-241	938	-2995	-1557	-423	-2061	954	-2103	-1286	-301	-1791	-26	-375	-717	-784	-1691	-2330	-1728	534
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
529(R)	524	-2098	-789	-146	-2504	-1729	1632	-2153	1229	-2054	-1204	-379	-1789	1328	2313	-719	-724	-1774	-2150	-1637	535
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
530(R)	-2957	-3022	-3318	-2735	-3796	-2998	-1968	-3912	-846	-3631	-3157	-2611	-3280	-1724	4058	-3026	-2913	-3650	-3096	-3185	536
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
531(R)	-1895	-2713	-2327	-1192	-3484	-2002	-481	-2856	2144	-2544	-1842	-1161	-2458	1393	3023	-1770	-1619	-2599	-2421	-2259	537
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
532(A)	2935	-1714	-553	857	-2769	-1546	-1218	-2333	-1106	-2591	-1873	-809	-2065	-934	-1502	-964	-1103	-1872	-2898	-2374	538
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
533(A)	1291	-1874	-176	1227	-2177	-1392	-109	-1909	277	-1891	-995	1134	-1522	1248	-228	-361	562	-1492	-2090	-1410	539
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
534(W)	-805	-687	-2591	-2028	138	-2236	-697	897	-1681	-421	141	-1646	-2282	-1369	-1627	-1315	636	-90	4478	1809	540
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
535(H)	-408	-1801	-274	1264	-2096	-1385	1508	-1822	1168	-1802	-899	-33	-1479	1381	-102	-303	595	221	-1996	-1339	541
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

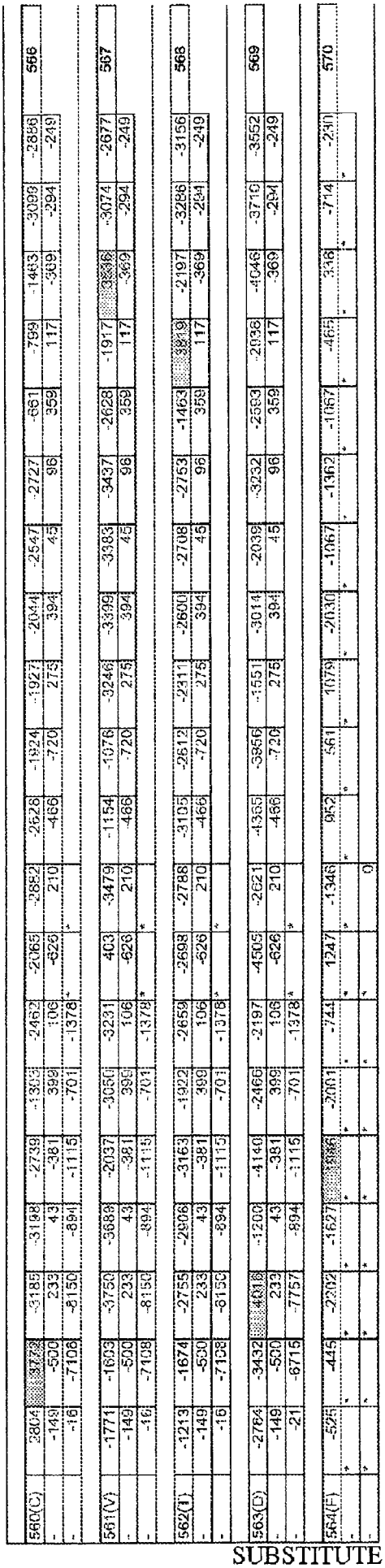
Table 12

536(Q)	-650	-1737	-627	-72	-1981	-1615	-209	-1625	1223	392	-866	-918	1222	2120	50	-598	-572	-1926	-1932	-1394	542
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
537(P)	-2931	-2878	-3420	-3708	-4181	-2925	-3468	-4621	-3859	-4490	-4165	-3491	-4225	-3781	-3695	-3182	-3279	-4087	-3594	-4064	543
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-324	-7108	-2368	-894	-1115	-701	-1378	*		*											
538(A)	2195	-924	-968	-546	-1397	-1356	-583	-812	-365	-1167	-487	-618	-1660	1324	-684	-483	-404	462	-1703	-1242	544
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-19	-6604	-7846	-894	-1115	-426	-1951	*		*											
539(P)	411	-1017	-1886	-1616	-1600	-1586	-1411	-962	-1408	495	-755	-1364	3158	-1323	-1577	-847	-765	-783	-2111	-1716	545
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
540(R)	-1612	-2397	-2037	-1033	-2897	-2352	-458	-2365	2184	665	-1520	-1051	-2334	-51	2602	-1545	-1395	-2143	-2262	-2014	546
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
541(Y)	712	-796	-2334	-1883	-370	-2028	-986	-143	-1607	-663	-131	-1587	-2243	-1383	-1656	-1178	-771	1114	-965	3479	547
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
542(T)	-527	-1689	1081	-27	-2315	-1979	-443	-2033	-151	-2081	-1218	-282	557	-41	-850	1128	2077	-1576	-2321	-1690	548
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
543(R)	-2957	-3022	-3318	-2736	-3796	-2998	-1968	-3912	-846	-3631	-3157	-2011	-3280	-1724	4656	-3026	-2913	-3650	-3096	-3165	549
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
544(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3952	-3748	-3779	-2839	-2981	-4004	-3668	-4222	550
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
545(V)	-1747	-1296	-4310	-3948	-1758	-4023	-3716	2215	-3813	-615	-540	-3705	-3860	-3670	-3887	-3339	-1741	3087	-3252	-2806	551
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
546(L)	-2871	-2457	-4231	-4103	-1033	-3803	-3165	-541	-3734	1136	-31	-3935	-3797	-3286	-3484	-3713	-2869	-1136	-2394	-2220	552
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											
547(A)	2404	-890	-1926	-1629	-1803	1275	-1415	-1282	-1490	392	-863	-1316	-1930	-1328	-1674	-654	-644	-952	-2187	-1810	553
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*		*											

Table 12

548(K)	-2620	-2961	-2461	-2046	-3743	-2791	-1570	-3603	3784	-3387	-2839	-2042	-3039	-1260	-465	-2604	-2536	-3331	-3001	-2988	554
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
549(Y)	-3621	-2707	-4176	-4424	2950	-4049	-394	-2539	-4002	-1942	-1987	-2749	-3933	-2854	-3451	-3299	-3499	-2690	349	4096	555
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
550(A)	3438	-1472	-2846	-3040	-3287	-1726	-2735	-2840	-3028	-3257	-2662	-2236	-2447	-2798	-2944	-1216	-1387	-2183	-3405	-3320	556
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
551(H)	-1741	-2627	-2076	-1046	-3303	-2401	2713	-2751	2478	-2476	-1755	-1061	-2375	-27	2379	-1621	-1497	-2477	-2379	-2161	557
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
552(L)	-1014	-876	-2956	-2408	-582	-2550	-1529	1721	-2079	2042	345	-2114	-2581	-1775	-2028	454	-980	286	-1414	-1096	558
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
553(V)	933	-842	-2818	-2467	-1542	-1870	-1890	154	-2226	-1095	-617	-1932	-2326	-1995	-2259	-1126	1070	2709	-2160	-1826	559
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
554(S)	-787	-1522	-1486	-1172	-2714	-1599	-1112	-2500	-433	-2563	-1791	-1116	-2067	-796	1351	2918	-989	-1943	-2648	-2234	560
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
555(S)	-326	-1010	-1779	-1541	-2691	-1234	-1500	-2386	-1456	-2584	-1749	-1228	1196	-1330	-1747	2386	1967	-1662	-2876	-2496	561
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
556(A)	3121	-934	-2489	-2561	-3081	-1203	-2295	-2766	-2533	-3080	-2234	-1669	-1953	-2234	-2533	936	-746	-1844	-3331	-3090	562
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
557(S)	-807	-1462	-2333	-2543	-3185	-1640	-2474	-3294	-2686	-3487	-2780	-1973	-2360	-2483	-2703	3468	-1316	-2413	-3310	-3025	563
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
558(R)	-586	-1873	-516	979	-2188	-1543	-123	-1869	1290	-353	-980	-202	-1622	314	1866	-491	782	-1495	-2024	-1439	564
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												
559(G)	-2594	-2690	-3304	-3623	-4328	3747	-3462	-4761	-3953	-4671	-4212	-3320	-3352	-3748	-3779	-2839	-2981	-4004	-3668	-4222	565
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*	*												

Table 12



560(C)	2804	3788	-3185	-3188	-2739	-1383	-2462	-2065	-2882	-2628	-1924	-1927	-2044	-2547	-2727	-661	-799	-1483	-3089	-2856	566
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7106	-8150	-894	-1115	-701	-1378	*													
-																					
561(V)	-1771	-1603	-3750	-3688	-2037	-3050	-3231	403	-3479	-1154	-1076	-3246	-3309	-3383	-3437	-2628	-1917	-3336	-3074	-2677	567
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*													
-																					
562(T)	-1213	-1674	-2755	-2806	-3163	-1922	-2659	-2698	-2788	-3105	-2612	-2311	-2600	-2708	-2753	-1463	-3819	-2197	-3286	-3156	568
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-16	-7108	-8150	-894	-1115	-701	-1378	*													
-																					
563(D)	-2784	-3432	-4016	-1200	-4140	-2466	-2197	-4505	-2621	-4365	-3856	-1511	-3014	-2039	-3232	-2583	-2036	-4046	-3716	-3552	569
-	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275	394	45	96	359	117	-369	-294	-249	
-	-21	-6715	-7757	-894	-1115	-701	-1378	*													
-																					
564(F)	-525	-445	-2202	-1627	-3886	-2061	-744	1247	-1346	952	561	1079	-2630	-1067	-1362	-1067	-465	338	-714	-230	570
-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
-																					

WHAT IS CLAIMED IS:

1. A recombinant host cell comprising:

(a) at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity; and

(b) (i) at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis; and/or

(ii) at least one heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis.

2. The recombinant host cell of claim 1, wherein said at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity:

(a) comprises a high copy number plasmid or a plasmid with a copy number that can be regulated; or

(b) is integrated at least once in the recombinant host cell DNA.

3. A recombinant host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity wherein said at least one heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated.

4. A recombinant host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity wherein said at least one heterologous polynucleotide is integrated at least once in the recombinant host cell DNA.

5. The recombinant host cell of claim 1 or 2, wherein said polypeptide affecting Fe-S cluster biosynthesis is encoded by a gene selected from the group consisting of the genes in Tables 8 and 9.

6. The recombinant host cell of claim 1 or 2, wherein said polypeptide affecting Fe-S cluster biosynthesis is encoded by a gene selected from the group consisting of the genes in Table 7.

7. The recombinant host cell of claim 5 or 6, wherein said polypeptide affecting Fe-S cluster biosynthesis is encoded by a gene selected from the group consisting of AFT1, AFT2, FRA2, GRX3, CCC1, and combinations thereof.

8. The recombinant host cell of claim 7, wherein said polypeptide is encoded by a polynucleotide that is constitutive mutant.

9. The recombinant host cell of claim 8, wherein said constitutive mutant is selected from the group consisting of AFT1 L99A, AFT1 L102A, AFT1 C291F, AFT1 C293F, and combinations thereof.

10. The recombinant host cell of claim 1 or 2, wherein said polypeptide affecting Fe-S cluster biosynthesis is AFT1, AFT2, FRA2, GRX3, or CCC1.

11. The recombinant host cell of claim 1 or 2, wherein said polypeptide affecting Fe-S cluster biosynthesis is AFT1, AFT2, FRA2, or CCC1.

12. The recombinant host cell of claim 1 or 2, wherein the at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of FRA2, GRX3, CCC1, and combinations thereof.

13. The recombinant host cell of claim 1 or 2, wherein the at least one heterologous polynucleotide encoding a polypeptide affecting Fe-S cluster biosynthesis is selected from the group consisting of AFT1, AFT2, and combinations thereof.

14. The recombinant host cell of claim 1, wherein said at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity is expressed in multiple copies.

15. The recombinant host cell of claim 14, wherein said at least one heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated.

16. The recombinant host cell of claim 14, wherein said at least one heterologous polynucleotide is integrated at least once in the recombinant host cell DNA.

17. The recombinant host cell of any one of claims 1,2, or 5-16, wherein said Fe-S cluster biosynthesis is increased compared to a recombinant host cell having endogenous Fe-S cluster biosynthesis.

18. The recombinant host cell of any one of claims 1-17, wherein said host cell is a yeast host cell.

19. The recombinant host cell of claim 18, wherein said yeast host cell is selected from the group consisting of Saccharomyces, Schizosaccharomyces, Hansenula, Candida, Kluyveromyces, Yarrowia, Issatchenkia and Pichia.

20. The recombinant host cell of any one of claims 1-19, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in the cytosol of the host cell.

21. The recombinant host cell of any one of claims 1-20, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of < 10-5 wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the Streptococcus mutans DHAD enzyme corresponding to SEQ ID NO:168.

22. The recombinant host cell of any one of claims 1-21, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence with at least about 90% identity to SEQ ID NO: 168 or SEQ ID NO: 232.

23. The recombinant host cell of any one of claims 1-22, wherein said polypeptide having dihydroxy-acid dehydratase activity has a specific activity selected from the group consisting of:

(a) greater than about 5-fold with respect to a control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity;

(b) greater than about 8-fold with respect to a control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity; and

(c) greater than about 10-fold with respect to a control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity.

24. The recombinant host cell of any one of claims 1-22, wherein said polypeptide having dihydroxy-acid dehydratase activity has a specific activity selected from the group consisting of:

(a) greater than about 0.25 U/mg;

(b) greater than about 0.3 U/mg;

(c) greater than about 0.5 U/mg;

(d) greater than about 1.0 U/mg;

(e) greater than about 1.5 U/mg;

(f) greater than about 2.0 U/mg;

(g) greater than about 3.0 U/mg;

(h) greater than about 4.0 U/mg;

(i) greater than about 5.0 U/mg;

(j) greater than about 6.0 U/mg;

(k) greater than about 7.0 U/mg;

(l) greater than about 8.0 U/mg;

(m) greater than about 9.0 U/mg;

(n) greater than about 10.0 U/mg;

(o) greater than about 20.0 U/mg; and

(p) greater than about 50.0 U/mg.

25. The recombinant host cell of any one of claims 1-24, wherein said recombinant host cell produces isobutanol.

26. The recombinant host cell of claim 25, wherein said recombinant host cell comprises an isobutanol biosynthetic pathway.

27. A method of making a product comprising:

(a) providing the recombinant host cell of any one of claims 1-24;

(b) contacting the recombinant host cell of (a) with a fermentable carbon substrate in a fermentation medium under conditions wherein said product is produced;

wherein the product is selected from the group consisting of branched chain amino acids, pantothenic acid, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutanol, and combinations thereof.

28. A method of making isobutanol comprising:

(a) providing the recombinant host cell of any one of claims 1-24;

(b) contacting the recombinant host cell of (a) with a fermentable carbon substrate in a fermentation medium under conditions wherein isobutanol is produced.

29. A method for the conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate comprising:

(a) providing the recombinant host of any one of claims 1-24; and

(b) growing the recombinant host cell of (a) under conditions where the 2,3-dihydroxyisovalerate is converted to α-ketoisovalerate,

wherein 2,3-dihydroxyisovalerate is converted to α-ketoisovalerate.

30. A method for increasing the specific activity of a heterologous polypeptide having dihydroxy-acid dehydratase activity in a recombinant host cell comprising:

(a) providing a recombinant host cell of any one of claims 1-24; and

(b) growing the recombinant host cell of (a) under conditions whereby the heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in functional form having a specific activity greater than the same host cell lacking said heterologous polypeptide.

31. A method for increasing the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising:

(a) providing a recombinant host cell of any one of claims 3-24; and

(b) growing the recombinant host cell of (a) under conditions whereby the flux in the Fe-S cluster biosynthesis pathway in the host cell is increased.

32. A method of increasing the activity of an Fe-S cluster requiring protein in a recombinant host cell comprising:

(a) providing a recombinant host cell comprising an Fe-S cluster requiring protein;

(b) changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis in said host cell; and

(c) growing the recombinant host cell of (b) under conditions whereby the activity of the Fe-S cluster requiring protein is increased.

33. The method of claim 32, wherein said increase in activity is an amount selected from the group consisting of:

(a) greater than about 10%;

(b) greater than about 20%;

(c) greater than about 30%;

(d) greater than about 40%;

(e) greater than about 50%;

(f) greater than about 60%;

(g) greater than about 70%;

(h) greater than about 80%;

(i) greater than about 90%; and

(j) greater than about 95%.

34. The method of claim 32, wherein said increase in activity is an amount selected from the group consisting of:

(a) greater than about 5-fold;

(b) greater than about 8-fold; and

(c) greater than about 10-fold.

35. A method for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising:

(a) changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis;

(b) measuring the activity of a heterologous Fe-S cluster requiring protein; and

(c) comparing the activity of the heterologous Fe-S cluster requiring protein measured in the presence of the changed expression or activity of a polypeptide of step (a) to the activity of the heterologous Fe-S cluster requiring protein measured in the absence of the changed expression or activity of a polypeptide of step (a),

wherein an increase in the activity of the heterologous Fe-S cluster requiring protein indicates an increase in the flux in said Fe-S cluster biosynthesis pathway.

36. A method for identifying polypeptides that increase the flux in an Fe-S cluster biosynthesis pathway in a host cell comprising:

(a) changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis;

(b) measuring the activity of a polypeptide having dihydroxy-acid dehydratase activity; and

(c) comparing the activity of the polypeptide having dihydroxy-acid dehydratase activity measured in the presence of the change in expression or activity of a polypeptide of step (a) to the activity of the polypeptide having dihydroxy-acid dehydratase activity measured in the absence of the change in expression or activity of a polypeptide of step (a),

wherein an increase in the activity of the polypeptide having dihydroxy-acid dehydratase activity indicates an increase in the flux in said Fe-S cluster biosynthesis pathway.

37. The method of any one of claims 30-36, wherein said changing the expression or activity of a polypeptide affecting Fe-S cluster biosynthesis comprises deleting, mutating, substituting, expressing, up-regulating, down-regulating, altering the cellular location, altering the state of the protein, and/or adding a cofactor.

38. The method of any one of claims 32-37, wherein the Fe-S cluster requiring protein has dihydroxy-acid dehydratase activity and wherein said Fe-S cluster requiring protein having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of < 10-5 wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the Streptococcus mutans DHAD enzyme corresponding to SEQ ID NO:168.

39. The method of any one of claims 32-38, wherein said polypeptide affecting Fe-S cluster biosynthesis is encoded by a gene selected from the group consisting of AFT1, AFT2, FRA2, GRX3, CCC1, and combinations thereof.

40. A recombinant host cell comprising at least one polynucleotide encoding a polypeptide identified by the methods of any one of claims 35-37.

41. The recombinant host cell of claim 40, wherein said host cell further comprises at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity.

42. The recombinant host cell of claim 41, wherein said heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity is expressed in multiple copies.

43. The recombinant host cell of claim 41, wherein said heterologous polynucleotide comprises a high copy number plasmid or a plasmid with a copy number that can be regulated.

44. The recombinant host cell of claim 41, wherein said heterologous polynucleotide is integrated at least once in the recombinant host cell DNA.

45. The method of claim 35 or 36, wherein said host cell is a yeast host cell.

46. The method of claim 45, wherein said yeast host cell is selected from the group consisting of Saccharomyces, Schizosaccharomyces, Hansenula, Candida, Kluyveromyces, Yarrowia, Issatchenkia, and Pichia.

47. The method of any one of claims 28-39, wherein said host cell is a yeast host cell.

48. The method of claim 47, wherein said yeast host cell is selected from the group consisting of Saccharomyces, Schizosaccharomyces, Hansenula, Candida, Kluyveromyces, Yarrowia, Issatchenkia, and Pichia.

49. The recombinant host cell of any one of claims 40-44, wherein said recombinant host cell is a yeast host cell.

50. The recombinant host cell of claim 49, wherein said yeast host cell is selected from the group consisting of Saccharomyces, Schizosaccharomyces, Hansenula, Candida, Kluyveromyces, Yarrowia, Issatchenkia, and Pichia.

51. The recombinant host cell of any one of claims 40-44 or 49-50, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity is expressed in the cytosol of the host cell.

52. The recombinant host cell of any one of claims 40-44 or 49-50, wherein said heterologous polypeptide having dihydroxy-acid dehydratase activity has an amino acid sequence that matches the Profile HMM of Table 12 with an E value of < 10-5 wherein the polypeptide further comprises all three conserved cysteines, corresponding to positions 56, 129, and 201 in the amino acids sequences of the Streptococcus mutans DHAD enzyme corresponding to SEQ ID NO:168.

53. The recombinant host cell of any one of claims 40-44 or 49-50, wherein said recombinant host cell produces a product selected from the group consisting of branched chain amino acids, pantothenic acid, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutanol, and combinations thereof.

54. The recombinant host cell of claim 53, wherein said recombinant host cell produces isobutanol.

55. The recombinant host cell of claim 54, wherein said recombinant host cell comprises an isobutanol biosynthetic pathway.

56. The recombinant host cell of any one of claims 1-22, wherein said polypeptide having dihydroxy-acid dehydratase activity has a specific activity selected from the group consisting of:

(a) greater than about 3-fold with respect to a control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity; and

(b) greater than about 6-fold with respect to a control host cell comprising at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity.

57. The method of claim 32, wherein said increase in activity is an amount selected from the group consisting of:

(a) greater than about 3-fold; and

(b) greater than about 6-fold.

58. The recombinant host cell of any one of claims 1-22, 40-44, or 49-50, wherein monomers of said polypeptide having dihydroxy-acid dehydratase activity have an Fe-S cluster loading selected from the group consisting of:

(a) at least about 10%;

(b) at least about 15%;

(c) at least about 20%;

(d) at least about 25%;

(e) at least about 30%;

(f) at least about 35%;

(g) at least about 40%;

(h) at least about 45%;

(i) at least about 50%;

(j) at least about 60%;

(k) at least about 70%;

(l) at least about 80%;

(m) at least about 90%; and

(n) at least about 95%.

59. The method of claim 29, wherein said conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate compared to a control host cell containing at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity is increased in an amount selected from the group consisting of:

(a) at least about 5%;

(b) at least about 10%;

(c) at least about 15%;

(d) at least about 20%;

(e) at least about 25%;

(f) at least about 30%;

(g) at least about 35%;

(h) at least about 40%;

(i) at least about 45%;

(j) at least about 50%;

(k) at least about 60%;

(l) at least about 70%;

(m) at least about 80%;

(n) at least about 90%; and

(o) at least about 95%.

60. The recombinant host cell of any one of claims 1, 2, or 5-26, wherein said polypeptide affecting Fe-S cluster biosynthesis is encoded by ARN1, ARN2, ATX1, CCC2, COT1, ENB1, FET3, FET5, FIT1, FIT2, FIT3, FRE1, FRE2, FRE3, FRE4, FRE5, FRE6, FTH1, FTR1, HMX1, SIT1, SMF3, TIS11,VHT1, AFT1, AFT2, AIM1, ARH1, ATM1, BUD32, CAD1, CCC1, CFD1, CIA1, CMK1, CTH1, CTI6, CYC8, DAP1, DRE2, ERV1, ESA1, FET4, FRA1, FRA2, GEF1, GGC1, GRX1, GRX2, GRX4, GRX5, HDA1, IBA57, ISA1, ISA2, ISU1, ISU2, JAC1, MGE1, MRS3, MRS4, MSN5, NAR1, NFS1, NFU1, NHP6a, NHP6b, PSE1, SMF1, SNF1, SNF2, SNF3, SNF4, SSQ1, TIM12, TUP1, NP_011911.1, VPS41, YAP5, YFH1, YRA1, ZPR1,

iscAnif, nifU, nifS, cysE1, cysE2, iscS, iscU, iscA, hscB, hscA, Fdx, sufS, sufE, cysE3, sufS2, iscA2, Nfu, nfuA, nfuV, nfu, sufA, sufB, sufC, sufD, sufE1, sufS2, or sufE2

61. A method of measuring the concentration of forms of polypeptide having dihydroxy-acid dehydratase activity, comprising:

(a) separating fractions containing said polypeptide from a crude extract using a first chromatographic procedure;

(b) separating fractions containing a form of said polypeptide from (a) using a second chromatographic procedure; and

(c) measuring the concentration of said forms of said polypeptide obtained in (b).

Figure 1A

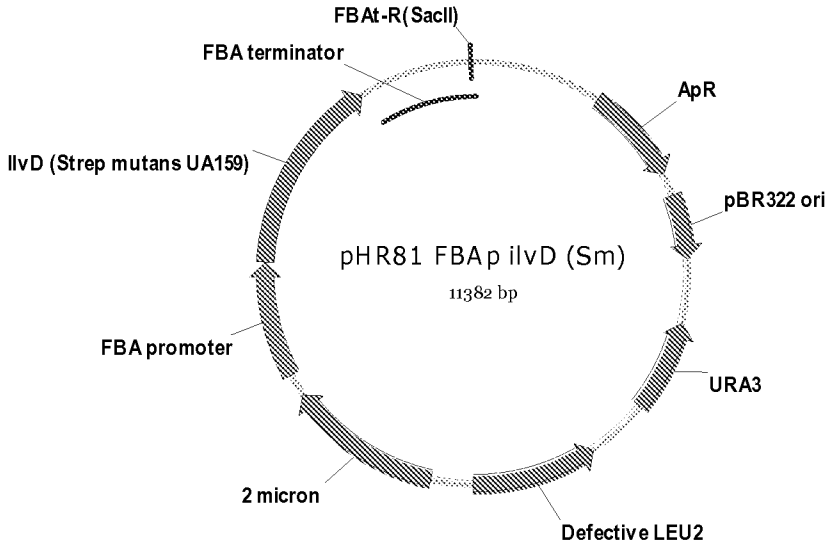


Figure 1B

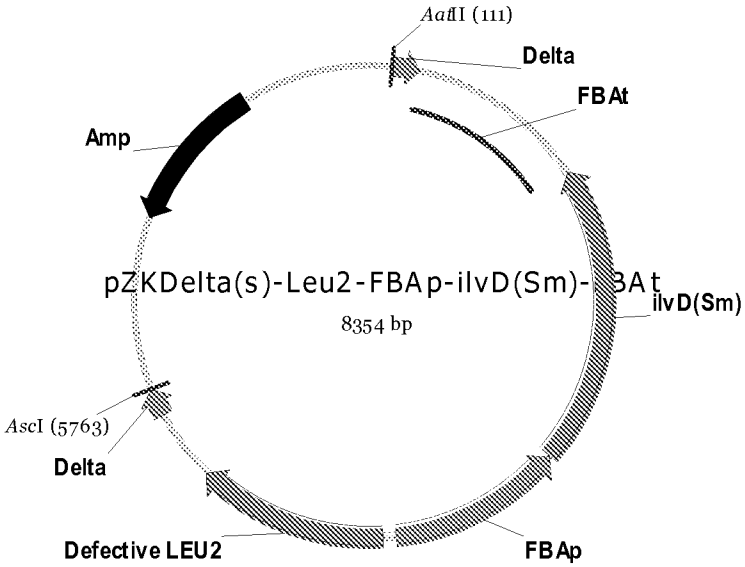
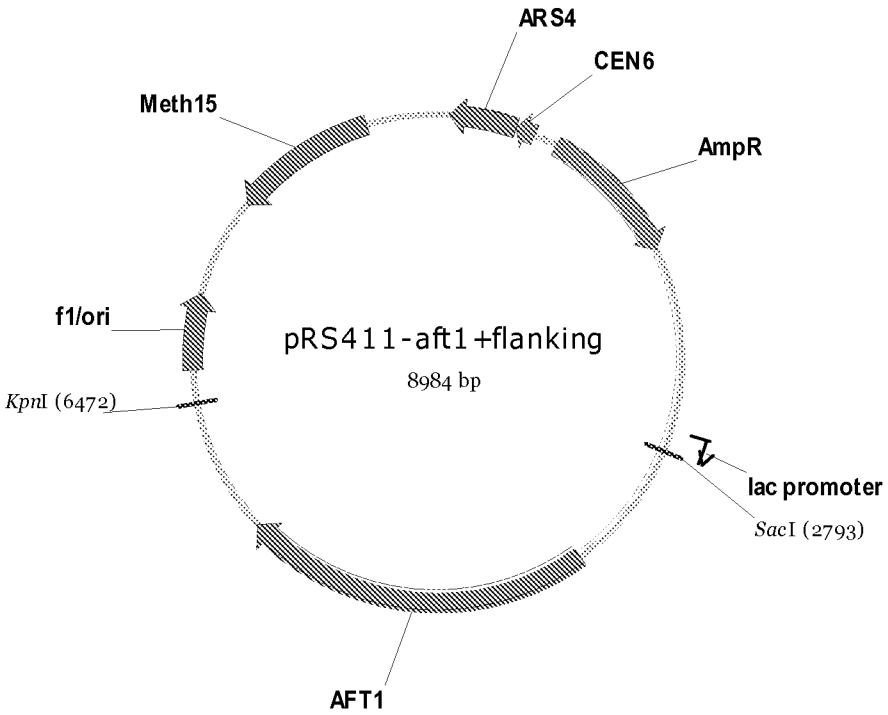


Figure 2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CEN6
Meth15
AmpR
f1/ori
pRS411-aft1+flanking
8984 bp
KpnI (6472)
lac promoter
SacI (2793)
AFT1

Figure 3

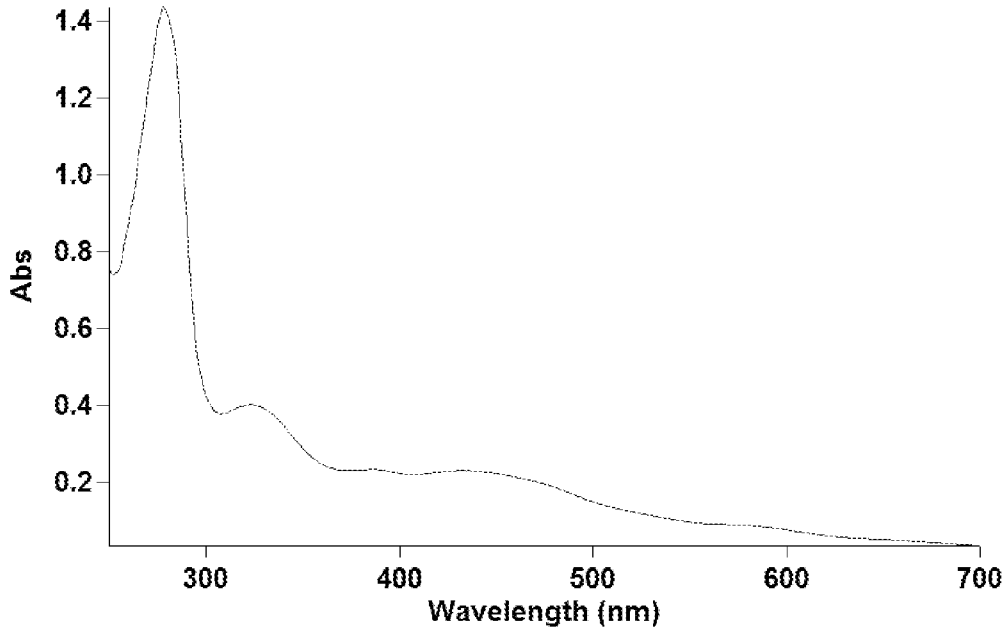


Figure 4

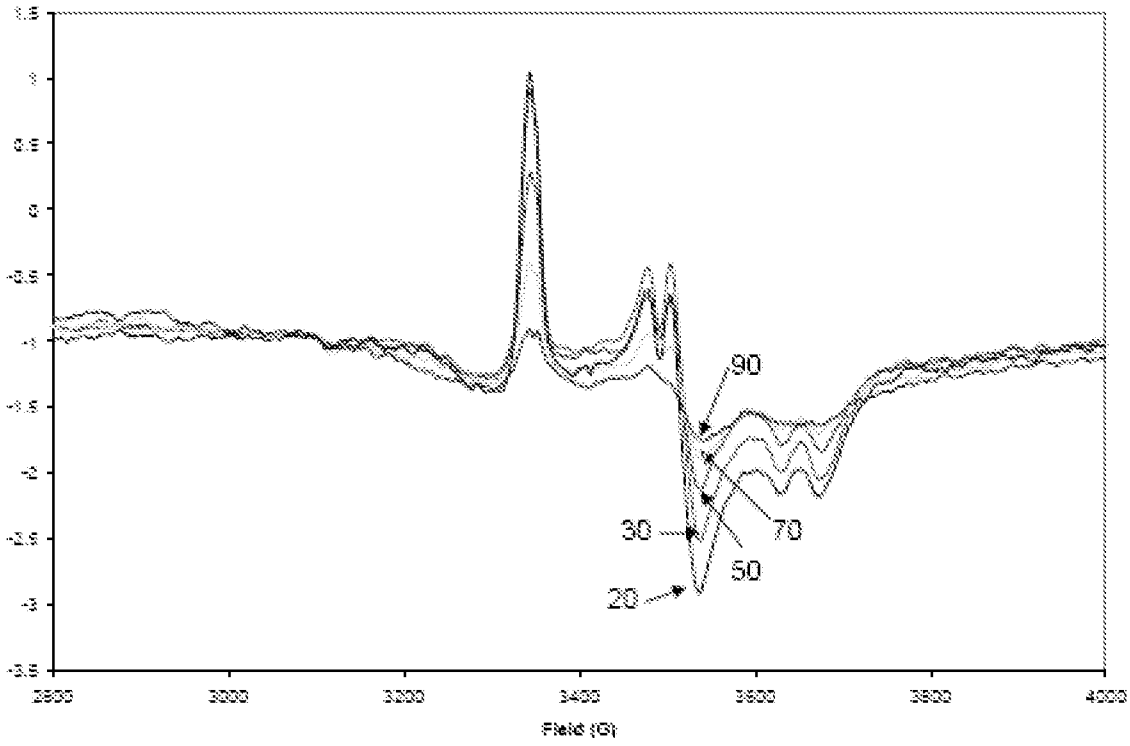


Figure 5

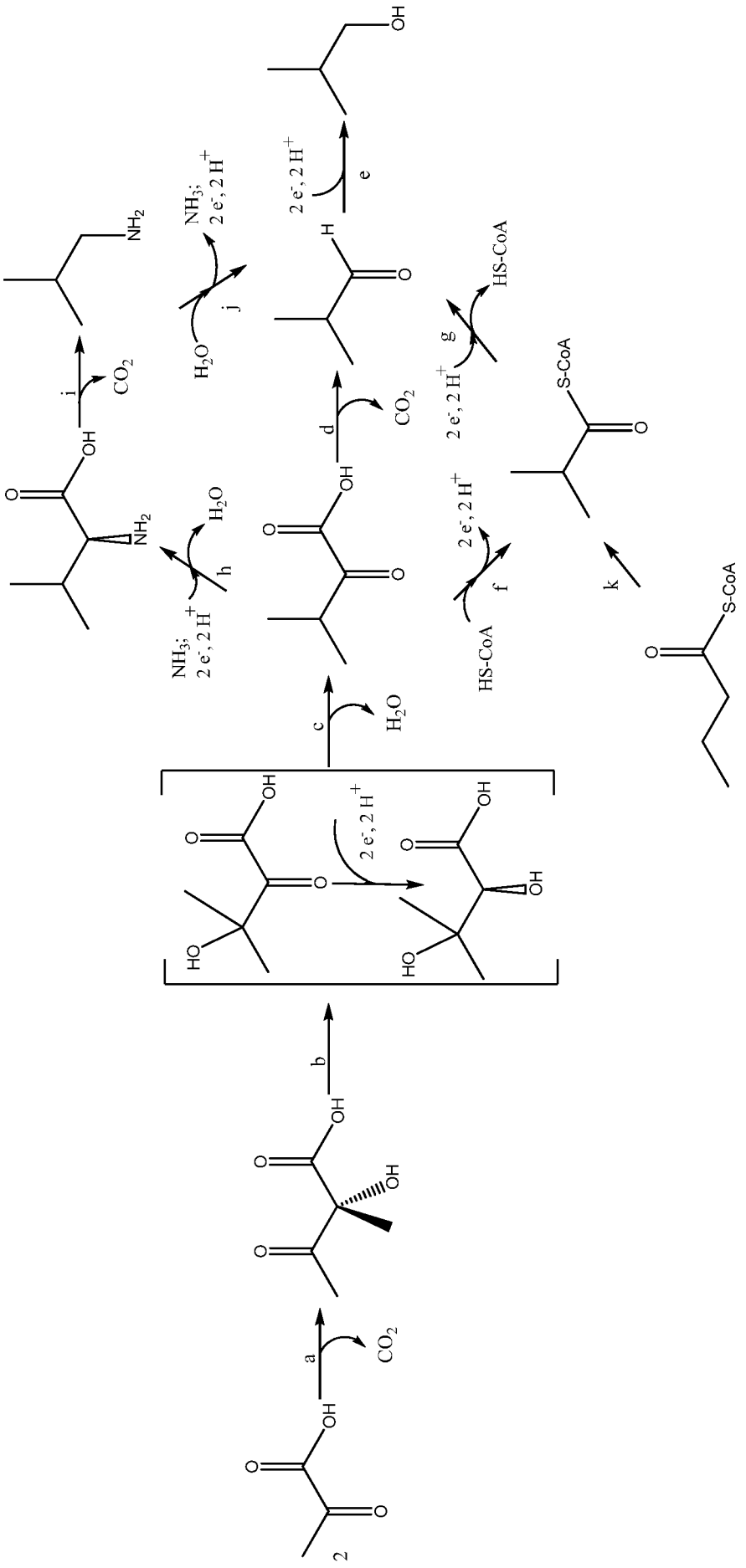

2
a
CO_2
b
c
H_2O
$2\,e^-, 2\,H^+$
d
CO_2
e
$2\,e^-, 2\,H^+$
f
$2\,e^-, 2\,H^+$
HS-CoA
g
HS-CoA
h
NH_3; $2\,e^-, 2\,H^+$
H_2O
i
CO_2
j
NH_3; $2\,e^-, 2\,H^+$
H_2O
k
S-CoA
S-CoA
OH
HO
NH_2
H

Figure 6A

Figure 6B

Figure 6C

$$\text{apo-ISU} + 2\ \text{IscS-Cys-S-S}^- + 2\ \text{Fe}^{2+} + 2\ \mathbf{NFU}^{\mathbf{red}}$$

$$\rightarrow \quad [2\text{Fe-2S}]\text{-ISU} + 2\ \text{IscS-Cys-S}^- + 2\ \mathbf{NFU}^{\mathbf{ox}}$$

Figure 7

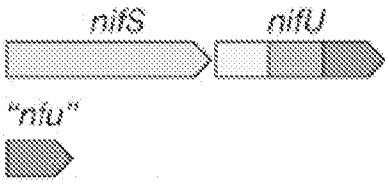


Figure 8

Figure 9

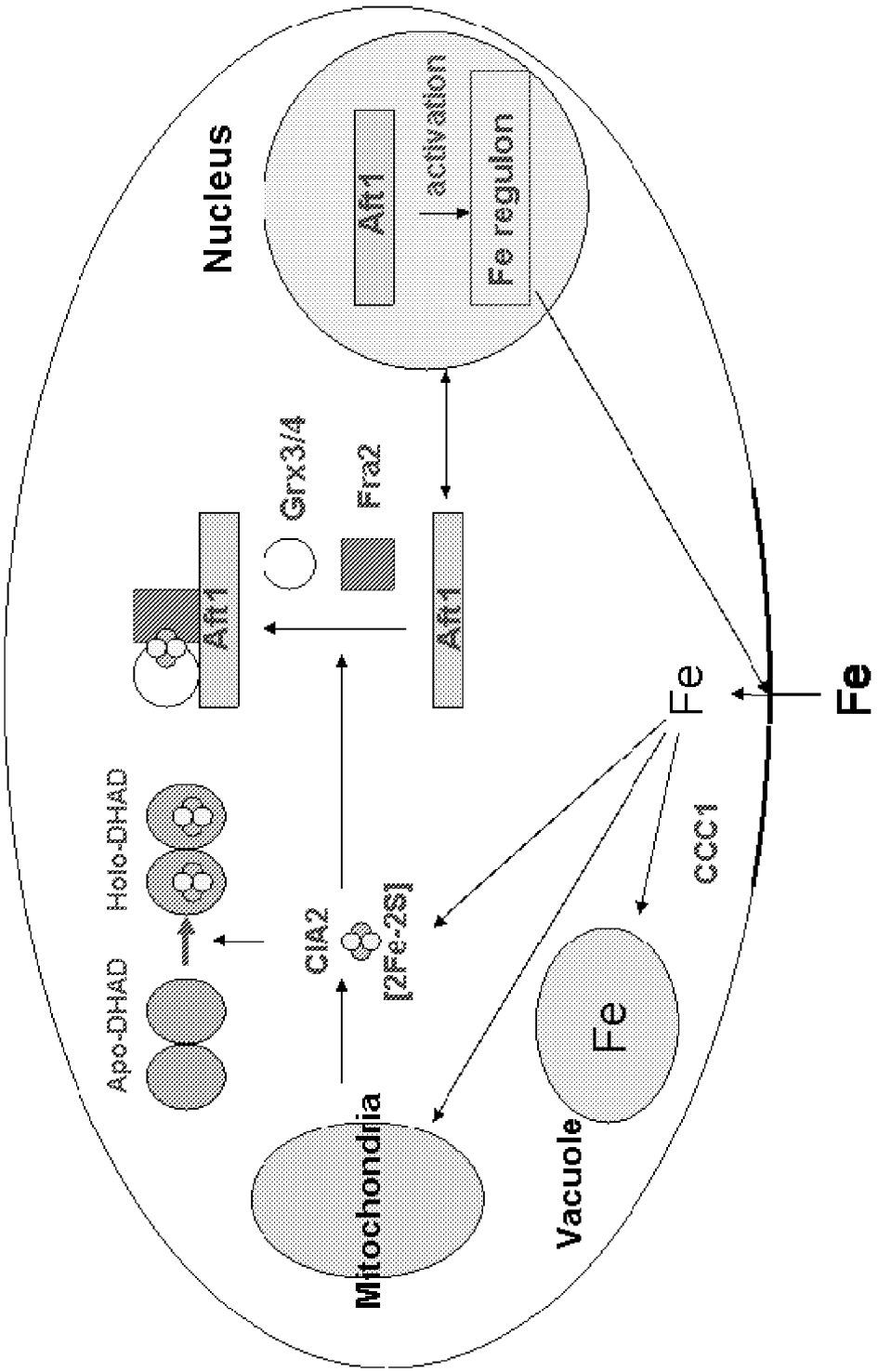
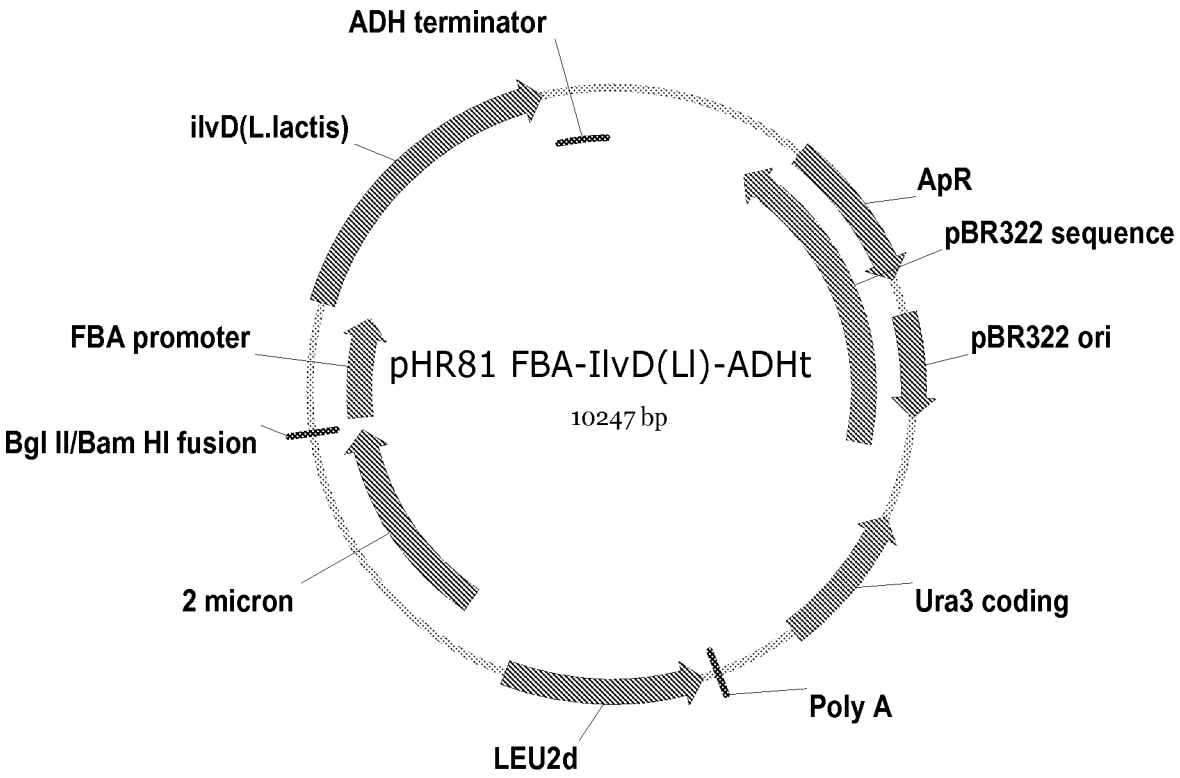

Nucleus
Aft1
activation
Fe regulon
Grx3/4
Fra2
Aft1
Aft1
Fe
Fe
ccc1
Holo-DHAD
Apo-DHAD
CIA2
[2Fe-2S]
Mitochondria
Vacuole
Fe

Figure 10

Figure 11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2 micron
Ura3 coding
Poly A
LEU2d