

(19) **United States**(12) **Patent Application Publication**

Li et al.

(10) **Pub. No.: US 2009/0163376 A1**(43) **Pub. Date: Jun. 25, 2009**(54) **KETOL-ACID REDUCTOISOMERASE USING NADH**(75) Inventors: **Yougen Li**, Lawrenceville, NJ (US); **Der-Ing Liao**, Wilmington, DE (US); **Mark J. Nelson**, Newark, DE (US); **Daniel P. Okeefe**, Ridley Park, PA (US)Correspondence Address:  
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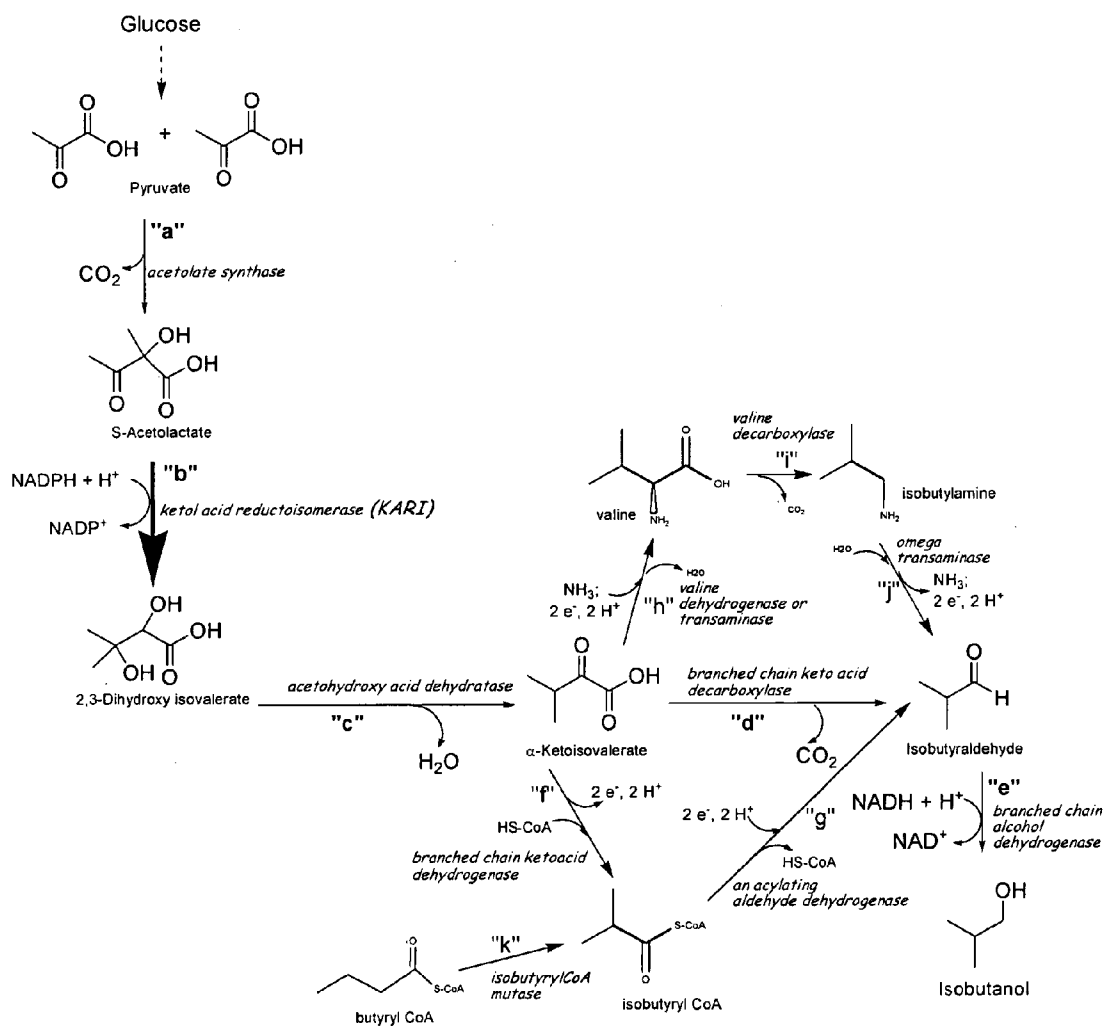
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**C12P 7/16** (2006.01)  
(52) **U.S. Cl.** ..... **506/9**; 435/233; 536/23.2; 435/325; 435/160(57) **ABSTRACT**Methods for the evolution of NADPH binding ketol-acid reductoisomerase enzymes to acquire NADH binding functionality are provided. Specific mutant ketol-acid reductoisomerase enzymes isolated from *Pseudomonas* that have undergone co-factor switching to bind NADH are described.

Figure 1

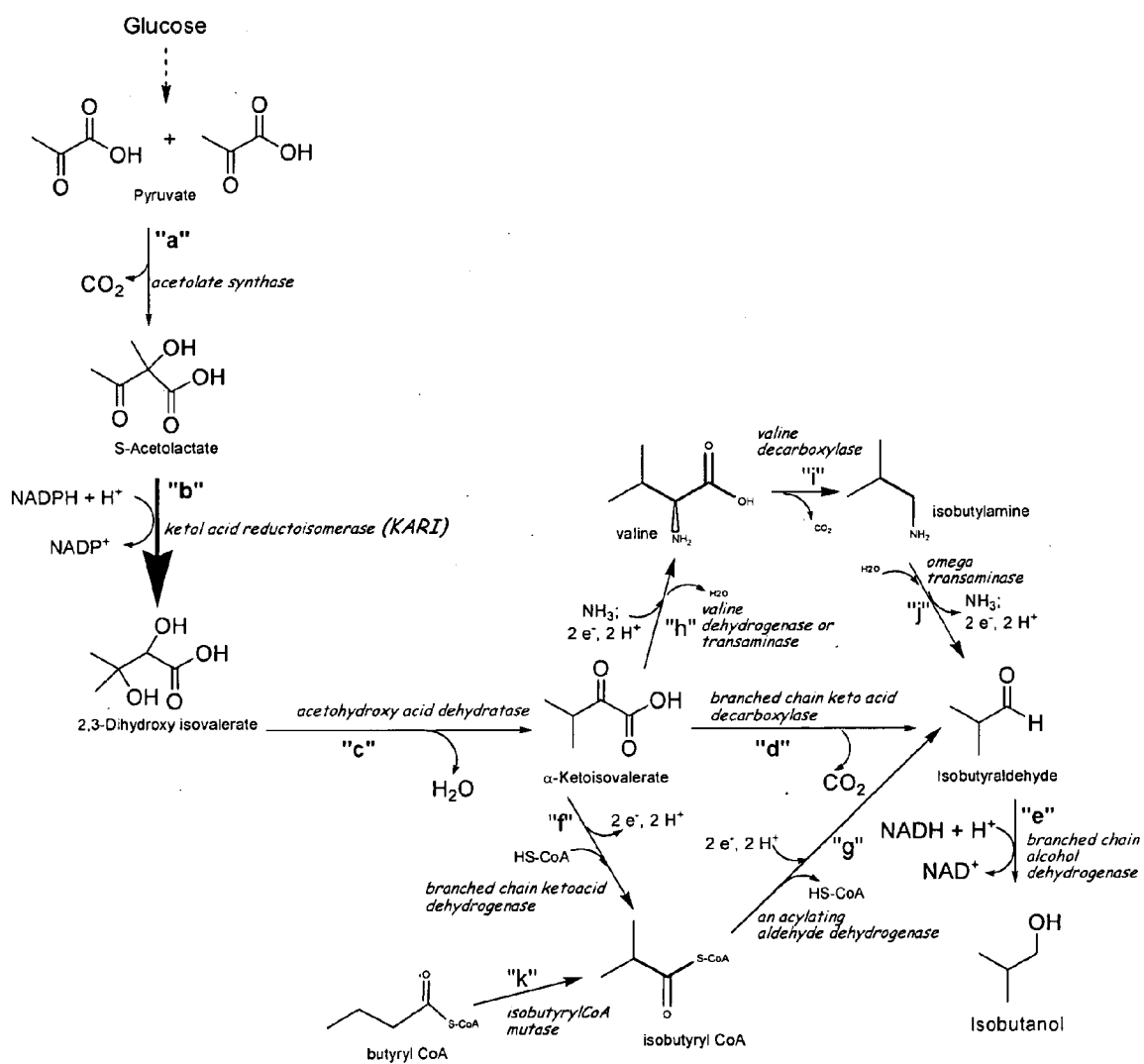


Figure 2

**a**

PF5-KARI	(44)	VGLR <b>R</b> KGS <b>S</b> AT <b>T</b> VAKA
PAO-KARI	(44)	VGL <b>R</b> SG <b>S</b> AT <b>T</b> VAKA
Spinach_KARI	(162)	IGL <b>R</b> KGS <b>S</b> NS <b>S</b> FAEA

**b**

		59		72
MMC5	(44)	VGLRKN <b>G</b> AS <b>W</b> ENAK		
MMS2	(44)	VGLRKN <b>G</b> AS <b>W</b> NNAK		
MVSB	(44)	VGLRKN <b>G</b> AS <b>W</b> ENAK		
PF5-ilvC	(44)	VGLRKGSATVAKAE		
KARI-S1	(44)	VGLRKN <b>G</b> AS <b>W</b> NKAV		
ilv5	(59)	IGVRKD <b>G</b> AS <b>W</b> KAAI		
KARI-D1	(48)	VGLEREGK <b>S</b> WELAK		
KARI-D2	(45)	IGLRRG <b>G</b> K <b>S</b> WELAT		
Consensus	(59)	VGLRKN <b>G</b> AS <b>W</b> E AK		

Figure 3

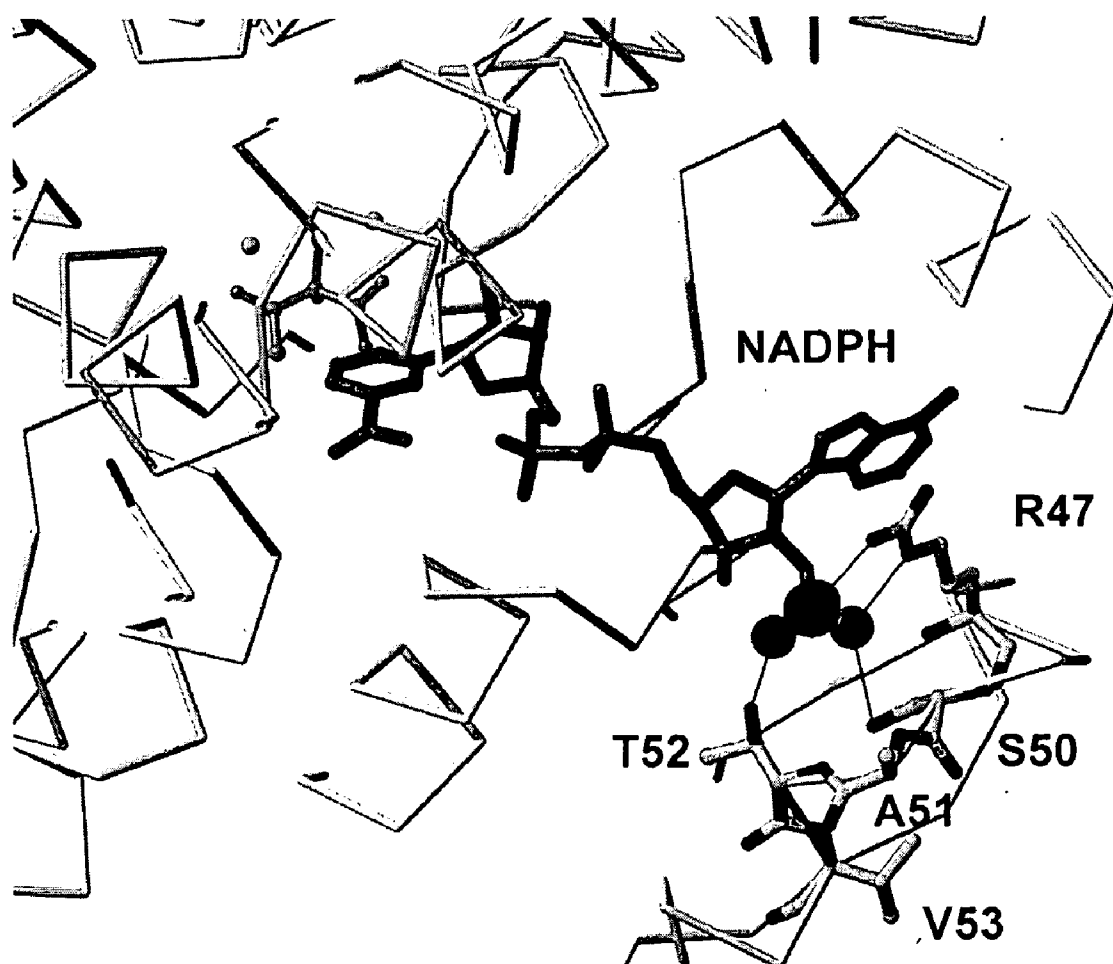




Figure 4

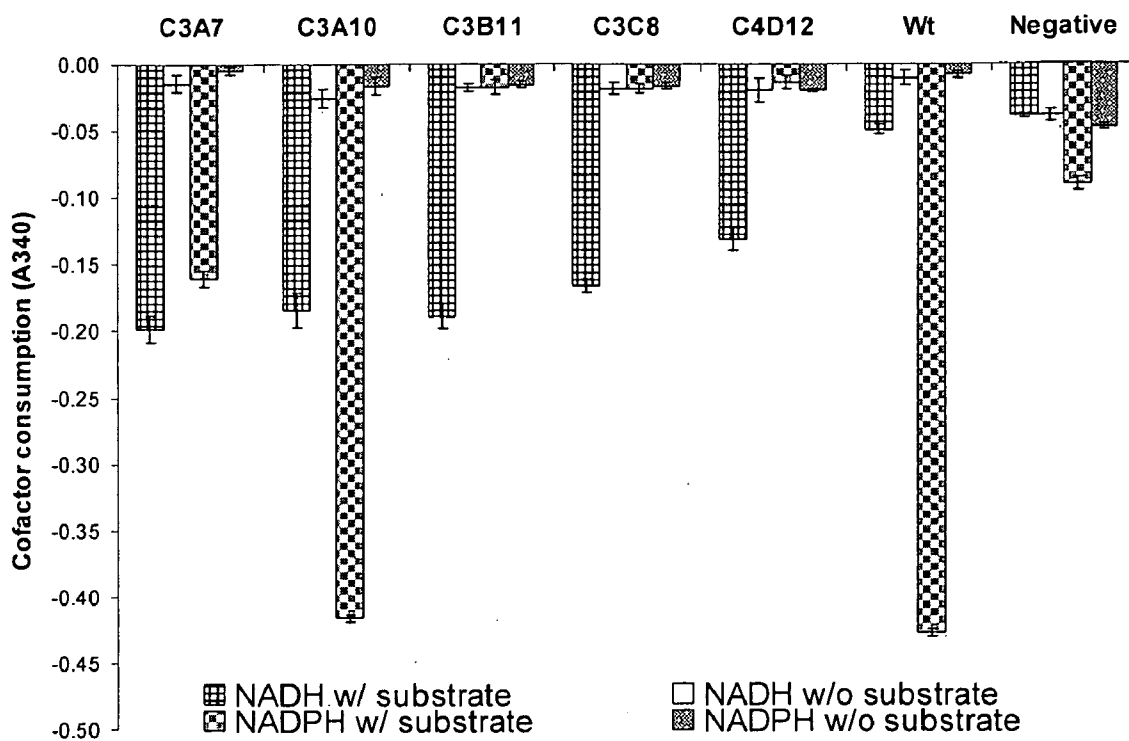


Figure 5

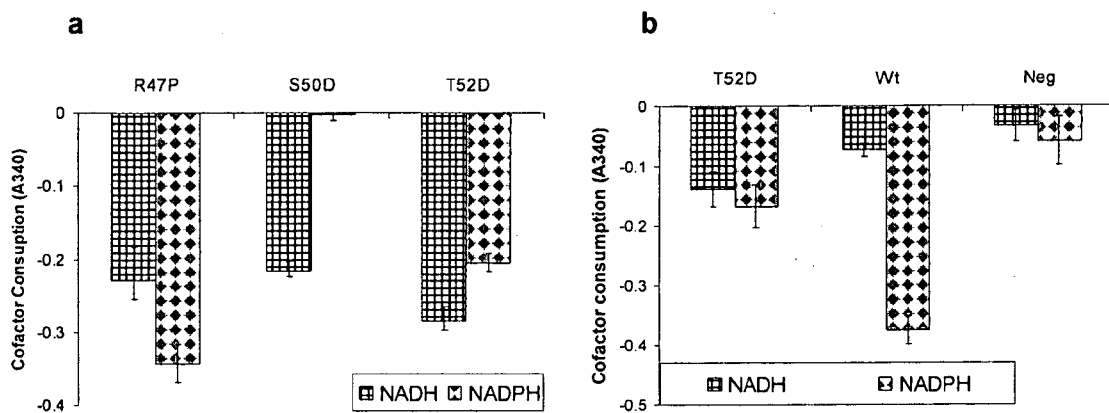


Figure 6

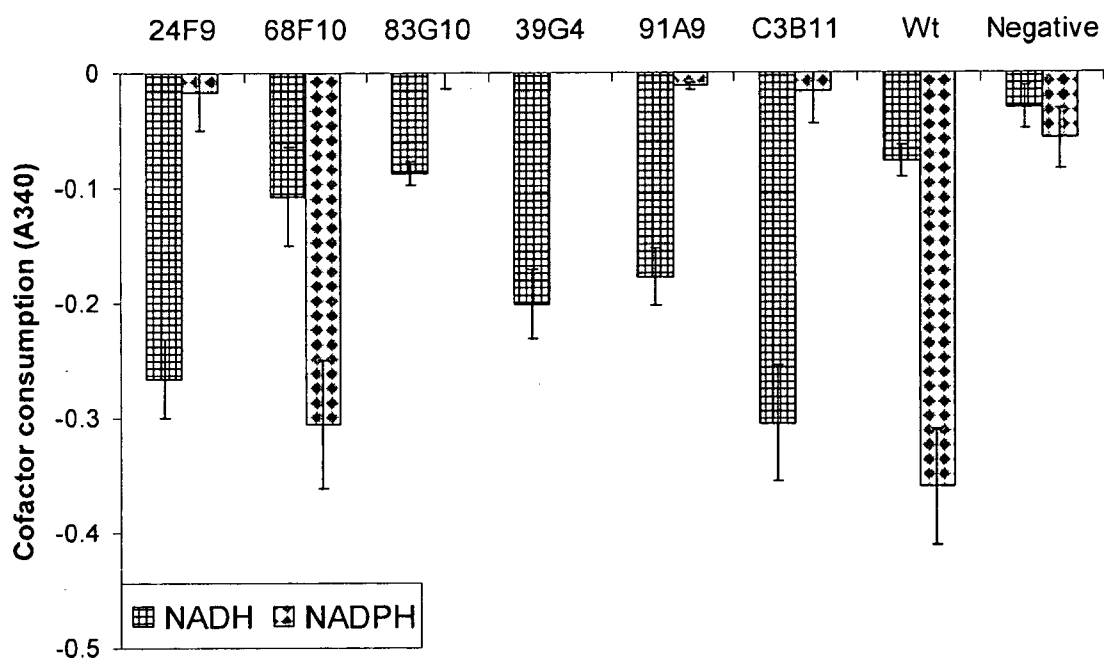


Figure 7

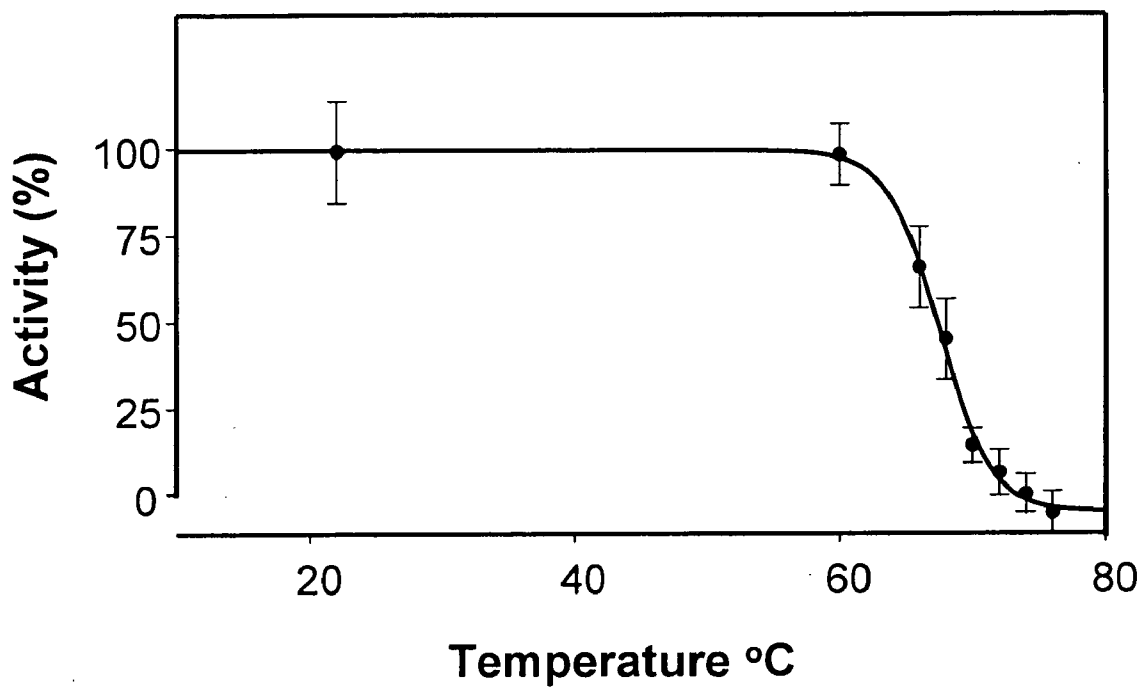
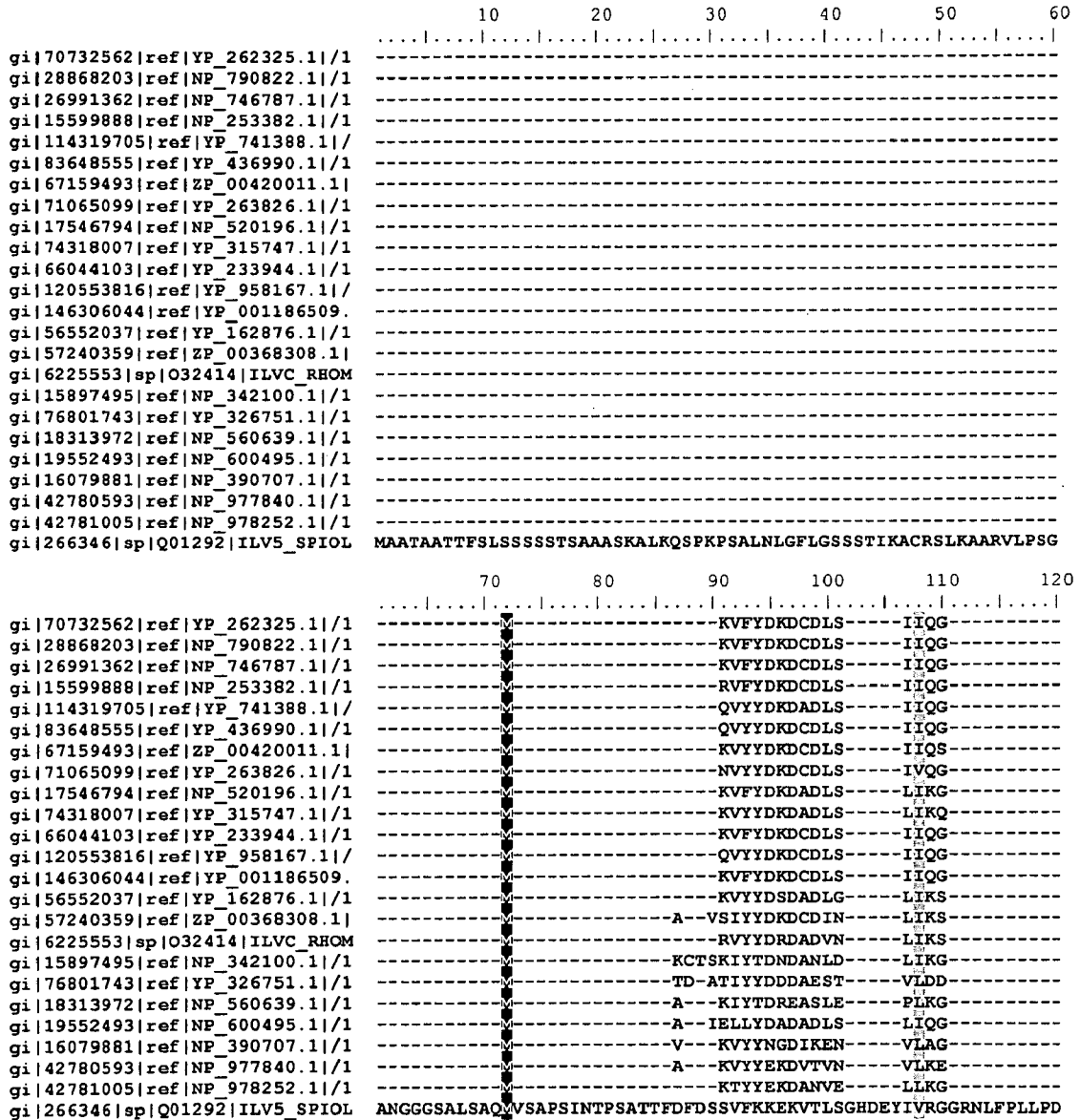


Figure 8

		51		100
ilvC1 B cereus	(18)	----K <b>K</b> V <b>A</b> I <b>I</b> G <b>Y</b> G <b>S</b> Q <b>G</b> H <b>A</b> H <b>A</b> Q <b>N</b> L <b>R</b> D-----N <b>G</b> F <b>D</b> V <b>V</b> V <b>G</b> L <b>R</b> K <b>G</b> -K <b>S</b> W <b>D</b> K <b>A</b>		
ilvC2 B cereus	(17)	----K <b>T</b> V <b>A</b> V <b>I</b> G <b>Y</b> G <b>S</b> Q <b>G</b> H <b>A</b> Q <b>A</b> Q <b>N</b> L <b>R</b> D-----S <b>G</b> V <b>E</b> V <b>V</b> V <b>G</b> V <b>R</b> P <b>G</b> -K <b>S</b> F <b>E</b> V <b>A</b>		
Spinach KARI	(51)	F <b>K</b> G <b>I</b> K <b>Q</b> I <b>G</b> V <b>I</b> G <b>W</b> G <b>S</b> Q <b>A</b> P <b>A</b> Q <b>A</b> Q <b>N</b> L <b>K</b> D <b>S</b> L <b>T</b> E <b>A</b> K <b>S</b> D <b>V</b> V <b>V</b> K <b>I</b> G <b>L</b> R <b>K</b> G <b>S</b> N <b>S</b> F <b>A</b> E <b>A</b>		
PAO KARI	(17)	----K <b>K</b> V <b>A</b> I <b>I</b> G <b>Y</b> G <b>S</b> Q <b>G</b> H <b>A</b> H <b>A</b> C <b>N</b> L <b>K</b> D-----S <b>G</b> V <b>D</b> V <b>T</b> V <b>G</b> L <b>R</b> S <b>G</b> S <b>A</b> T <b>V</b> A <b>K</b> A		
PF5 KARI	(17)	----K <b>K</b> V <b>A</b> I <b>I</b> G <b>Y</b> G <b>S</b> Q <b>G</b> H <b>A</b> Q <b>A</b> C <b>N</b> L <b>K</b> D-----S <b>G</b> V <b>D</b> V <b>T</b> V <b>G</b> L <b>R</b> K <b>G</b> S <b>A</b> T <b>V</b> A <b>K</b> A		
		101		150
ilvC1 B cereus	(57)	K <b>E</b> D <b>G</b> E <b>S</b> -----V <b>T</b> V <b>A</b> E <b>A</b> A <b>K</b> Q <b>A</b> D <b>V</b> V <b>M</b> I <b>L</b> L <b>P</b> D <b>E</b> L <b>Q</b> P <b>E</b> V <b>E</b> A <b>E</b> I <b>A</b> P <b>N</b> L <b>Q</b> A <b>G</b> N		
ilvC2 B cereus	(56)	K <b>T</b> D <b>G</b> E <b>E</b> -----V <b>M</b> S <b>V</b> S <b>E</b> A <b>V</b> R <b>T</b> A <b>Q</b> V <b>V</b> Q <b>M</b> L <b>L</b> P <b>D</b> E <b>Q</b> Q <b>A</b> H <b>V</b> Y <b>K</b> A <b>G</b> V <b>E</b> E <b>N</b> L <b>R</b> E <b>G</b> Q		
Spinach KARI	(101)	R <b>A</b> A <b>G</b> E <b>S</b> E <b>E</b> N <b>G</b> T <b>L</b> G <b>D</b> M <b>W</b> E <b>T</b> I <b>S</b> G <b>S</b> D <b>L</b> V <b>L</b> L <b>L</b> L <b>S</b> D <b>S</b> A <b>Q</b> A <b>D</b> N <b>Y</b> E <b>K</b> V <b>F</b> S <b>H</b> M <b>K</b> -P <b>N</b> S		
PAO KARI	(57)	E <b>A</b> H <b>G</b> L <b>K</b> -----V <b>A</b> D <b>V</b> K <b>T</b> A <b>V</b> A <b>A</b> A <b>D</b> V <b>V</b> M <b>I</b> L <b>L</b> P <b>D</b> E <b>F</b> Q <b>G</b> R <b>L</b> Y <b>K</b> E <b>E</b> I <b>E</b> P <b>N</b> L <b>K</b> K <b>G</b> A		
PF5 KARI	(57)	E <b>A</b> H <b>G</b> L <b>K</b> -----V <b>T</b> D <b>V</b> A <b>A</b> A <b>V</b> A <b>G</b> A <b>D</b> L <b>V</b> M <b>I</b> L <b>L</b> P <b>D</b> E <b>F</b> Q <b>S</b> Q <b>L</b> Y <b>K</b> N <b>E</b> I <b>E</b> P <b>N</b> I <b>K</b> K <b>G</b> A		
		151		200
ilvC1 B cereus	(102)	S <b>L</b> V <b>F</b> A <b>H</b> G <b>F</b> N <b>V</b> H <b>F</b> D <b>Q</b> V <b>K</b> ---P <b>P</b> A <b>N</b> V <b>D</b> V <b>F</b> L <b>V</b> A <b>P</b> K <b>G</b> P <b>G</b> H <b>L</b> V <b>R</b> R <b>T</b> F <b>S</b> E <b>G</b> -----		
ilvC2 B cereus	(101)	M <b>L</b> L <b>F</b> S <b>H</b> G <b>F</b> N <b>I</b> H <b>F</b> G <b>Q</b> I <b>N</b> ---P <b>P</b> S <b>Y</b> V <b>D</b> V <b>A</b> M <b>V</b> A <b>P</b> K <b>S</b> P <b>G</b> H <b>L</b> V <b>R</b> R <b>V</b> F <b>Q</b> E <b>G</b> -----		
Spinach KARI	(150)	I <b>L</b> G <b>L</b> S <b>H</b> G <b>F</b> L <b>L</b> G <b>H</b> L <b>Q</b> S <b>L</b> G <b>Q</b> D <b>F</b> P <b>K</b> N <b>I</b> S <b>V</b> I <b>A</b> V <b>C</b> P <b>K</b> G <b>M</b> G <b>P</b> S <b>V</b> R <b>R</b> L <b>V</b> Y <b>Q</b> G <b>K</b> E <b>V</b> N <b>G</b>		
PAO KARI	(102)	T <b>L</b> A <b>F</b> A <b>H</b> G <b>F</b> S <b>I</b> H <b>Y</b> N <b>Q</b> V <b>V</b> ---P <b>R</b> A <b>D</b> L <b>D</b> V <b>I</b> M <b>I</b> A <b>P</b> K <b>A</b> P <b>G</b> H <b>T</b> V <b>R</b> S <b>E</b> F <b>V</b> K <b>G</b> -----		
PF5 KARI	(102)	T <b>L</b> A <b>F</b> S <b>H</b> G <b>F</b> A <b>I</b> H <b>Y</b> N <b>Q</b> V <b>V</b> ---P <b>R</b> A <b>D</b> L <b>D</b> V <b>I</b> M <b>I</b> A <b>P</b> K <b>A</b> P <b>G</b> H <b>T</b> V <b>R</b> S <b>E</b> F <b>V</b> K <b>G</b> -----		
		201		250
ilvC1 B cereus	(144)	G <b>A</b> V <b>P</b> A <b>L</b> F <b>A</b> V <b>Y</b> Q <b>D</b> A <b>T</b> G <b>V</b> A <b>T</b> E <b>K</b> A <b>L</b> S <b>Y</b> A <b>D</b> G <b>I</b> G <b>A</b> T <b>R</b> A <b>G</b> V <b>L</b> E <b>T</b> T <b>F</b> K <b>E</b> E <b>T</b> E <b>T</b> D <b>L</b> F <b>G</b>		
ilvC2 B cereus	(143)	N <b>G</b> V <b>P</b> A <b>L</b> V <b>A</b> V <b>H</b> Q <b>D</b> A <b>T</b> G <b>T</b> A <b>L</b> H <b>V</b> A <b>L</b> A <b>Y</b> A <b>K</b> G <b>V</b> G <b>C</b> T <b>R</b> A <b>G</b> V <b>I</b> E <b>T</b> T <b>F</b> Q <b>E</b> E <b>T</b> E <b>T</b> D <b>L</b> F <b>G</b>		
Spinach KARI	(200)	A <b>G</b> I <b>N</b> S <b>S</b> F <b>A</b> V <b>H</b> Q <b>D</b> V <b>D</b> G <b>R</b> A <b>T</b> D <b>V</b> A <b>L</b> G <b>W</b> S <b>I</b> A <b>L</b> G--S <b>P</b> F <b>T</b> F <b>A</b> T <b>T</b> L <b>E</b> Q <b>E</b> Y <b>K</b> S <b>D</b> I <b>F</b> G		
PAO KARI	(144)	G <b>G</b> I <b>P</b> D <b>L</b> I <b>A</b> I <b>Y</b> Q <b>D</b> A <b>S</b> G <b>N</b> A <b>K</b> N <b>V</b> A <b>L</b> S <b>Y</b> A <b>C</b> G <b>V</b> G <b>G</b> G <b>R</b> T <b>G</b> I <b>I</b> E <b>T</b> T <b>F</b> K <b>D</b> E <b>T</b> E <b>T</b> D <b>L</b> F <b>G</b>		
PF5 KARI	(144)	G <b>G</b> I <b>P</b> D <b>L</b> I <b>A</b> I <b>Y</b> Q <b>D</b> A <b>S</b> G <b>N</b> A <b>K</b> N <b>V</b> A <b>L</b> S <b>Y</b> A <b>A</b> G <b>V</b> G <b>G</b> G <b>R</b> T <b>G</b> I <b>I</b> E <b>T</b> T <b>F</b> K <b>D</b> E <b>T</b> E <b>T</b> D <b>L</b> F <b>G</b>		

Figure 9



130 140 150 160 170 180

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GXGX (G/A)

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gi|26991362|ref|NP_746787.1|/1 A---DLDVIMLAPKAPGHTVRSEEVKGGIPDLIAIYODASGNKQNVALSYSAGV
gi|15599888|ref|NP_253382.1|/1 A---DLDVIMLAPKAPGHTVRSEEVKGGIPDLIAIYODASGNKQNVALSYSAGV
gi|114319705|ref|YP_741388.1|/1 A---DLDVIMVAPKPGCHLVRSTYVEGGVPSLIAIHODATGKARDIALSYASAN
gi|83648555|ref|YP_436990.1|/1 A---DLDVIMLAPKAPGHTVRSEEVKGGIPDLIAIYODASGNKQNVALSYSAGV
gi|67159493|ref|ZP_00420011.1| A---DLDVIMLAPKAPGHTVRSEEVKGGIPDLIAIYODASGNKQNVALSYSAGV
gi|71065099|ref|YP_263826.1|/1 S---DLDVIMVAPKAPGHTVRSEFAKGGIPDLIAIYODASGQAKOLALSYSAGV
gi|17546794|ref|NP_520196.1|/1 A---DLDVIMVAPKAPGHTVRGTYAQGGVPHLIAVHODKSGSARDIALSYATAN
gi|74318007|ref|YP_315747.1|/1 A---DLDVIMLAPKAPGHTVRSEEVKGGIPDLIAIYODASGNKQNVALSYSAGV
gi|66044103|ref|YP_233944.1|/1 A---DLDVIMLAPKAPGHTVRSEEVKGGIPDLIAIYODASGNKQNVALSYSAGV
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gi|146306044|ref|YP_001186509. A---DLDVIMLAPKAPGHTVRSEEVKGGIPDLIAIYODASGNKQNVALSYSAGV
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gi|57240359|ref|ZP_00368308.1| K---DLDVIMLAPKAPGHTVRSEEVKGGIPDLIAIYODASGNKQNVALSYSAGV
gi|6225553|sp|O32414|ILVC_RHOM A---DLDVIMVAPKPGCHTVRGEYLKGGVPCLVAVNPNPTGNALELALSYSASAI
gi|15897495|ref|NP_342100.1|/1 K---DSDVIMLAPKPGCHLVRREYKAGGGVPALVAVHODVSGTALHKAPAIKAGI
gi|76801743|ref|YP_326751.1|/1 E---DVMVIMVAPKSPCHLVRREYKAGGGVPCLVAVNPNPTGNALELALSYSASAI
gi|18313972|ref|NP_560639.1|/1 K---NIDVIMVAPKAPKAVREYLAGRGVPALVAVYODVSGSALKYALALAKGI
gi|19552493|ref|NP_600495.1|/1 D---DIIVGMVAPKPGCHLVRREYKAGGGVPALVAVHODVSGTALHKAPAIKAGI
gi|16079881|ref|NP_390707.1|/1 A---DNDVIMVAPKPGCHLVRRTYEQAGVPALFAVYODVSGEARDKALAYSAGI
gi|42780593|ref|NP_977840.1|/1 A---NVDVIMVAPKPGCHLVRRTYEQAGVPALFAVYODATGVATEKALSADGI
gi|42781005|ref|NP_978252.1|/1 S---YVDVIMVAPKSPCHLVRRTYEQAGVPALVAVHODATGTLHVADAYSAGV
gi|266346|sp|Q01292|ILV5_SPIO LDFPKNISVIAVCPKMGPSVRRLYVQKEVNGAGINSFAVHODVDRADVALGWSIAL

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310 320 330 340 350 360

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gi|28868203|ref|NP_790822.1|/1 GGGRTGIIETTFKDETDLDFGGEQAVLCCGTVELVKAGFETLVEAGYAPEMAYFECLHEL
gi|26991362|ref|NP_746787.1|/1 GGGRTGIIETTFKDETDLDFGGEQAVLCCGTVELVKAGFETLVEAGYAPEMAYFECLHEL
gi|15599888|ref|NP_253382.1|/1 GGGRTGIIETTFKDETDLDFGGEQAVLCCGTVELVKAGFETLVEAGYAPEMAYFECLHEL
gi|114319705|ref|YP_741388.1|/1 GGRAGVIEISFREBETDLEFGGEQAVLCCGITSLIQAGFETLVEAGYAPEMAYFECLHET
gi|83648555|ref|YP_436990.1|/1 GGGRTGIIETTFKDETDLDFGGEQAVLCCGAVELVKAGFETLVEAGYAPEMAYFECLHEL
gi|67159493|ref|ZP_00420011.1| GGGRTGIIETTFKDETDLDFGGEQAVLCCGCVLKVAGFETLVEAGYAPEMAYFECLHEL
gi|71065099|ref|YP_263826.1|/1 GGRSGIIEITFKDETDLDFGGEQAVLCCGAVELVKMGFETLVEAGYAPEMAYFECLHEL
gi|17546794|ref|NP_520196.1|/1 GGRAGIIEISFREBETDLEFGGEQAVLCCGTVELVKAGFETLVEAGYAPEMAYFECLHEL
gi|74318007|ref|YP_315747.1|/1 GGGRTGIIETTFKDETDLDFGGEQAVLCCGAVELVKAGFETLVEAGYAPEMAYFECLHEL
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gi|120553816|ref|YP_958167.1|/ GGGRTGIIETTFKDETDLDFGGEQAVLCCGAVELVKAGFETLVEAGYAPEMAYFECLHEL
gi|146306044|ref|YP_001186509. GGGRTGIIETTFKDETDLDFGGEQAVLCCGTVELVKAGFETLVEAGYAPEMAYFECLHEL
gi|56552037|ref|YP_162876.1|/1 GGRSGVIEISFREBETDLEFGGEQAVLCCGLTALITAGFETLVEAGYAPEMAYFECLHEM
gi|57240359|ref|ZP_00368308.1| GGGRTGIIETTFKDETDLDFGGEQAVLCCGLSALIQAGFETLVEAGYAPEMAYFECLHEM
gi|6225553|sp|O32414|ILVC_RHOM GGRSGIIEISFREBETDLEFGGEQAVLCCGLSKLIQYGFETLVEAGYAPEMAYFECLHEV
gi|15897495|ref|NP_342100.1|/1 GATRAGVIEISFREBETDLEFGGEQAVLCCGIMELMRAAFETLVEAGYAPEMAYFECLINEL
gi|76801743|ref|YP_326751.1|/1 GCTRAGVIEISFREBETDLEFGGEQAVLCCGVTSLVKTYGTYETLVEAGYAPEMAYFECLINEL
gi|18313972|ref|NP_560639.1|/1 GATRAGVIEISFREBETDLEFGGEQAVLCCGIMELMRAAFETLVEAGYAPEMAYFECLINEL
gi|19552493|ref|NP_600495.1|/1 GGARAGVIEISFREBETDLEFGGEQAVLCCGTEELVKVGFVETLVEAGYAPEMAYFECLHEL
gi|16079881|ref|NP_390707.1|/1 GGARAGVIEISFREBETDLEFGGEQAVLCCGLSALVKAGFETLVEAGYAPEMAYFECLHEL
gi|42780593|ref|NP_977840.1|/1 GATRAGVIEISFREBETDLEFGGEQAVLCCGVTVLKVAGFETLVEAGYAPEMAYFECLHEL
gi|42781005|ref|NP_978252.1|/1 GCTRAGVIEISFREBETDLEFGGEQAVLCCGVTVLKVAGFETLVEAGYAPEMAYFECLHEL
gi|266346|sp|Q01292|ILV5_SPIO GSPF--TFATLLEQEKSDIFGGERGILLCAVHGIVECLFRYTESGMSEDLAYKNTVECI

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370 380 390 400 410 420

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gi|28868203|ref|NP_790822.1|/1 K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGPEVINAESRQAMRNALKRITODGEYAKMF
gi|26991362|ref|NP_746787.1|/1 K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGPEVINAEESRKAMRNALKRITODGEYAKMF
gi|15599888|ref|NP_253382.1|/1 K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGPEVINAEESRAAMRNALKRITODGEYAKMF
gi|114319705|ref|YP_741388.1|/1 K-EVDLLEYQCGCIANMRYSTNTAEYGDFTRGPRVINEESREAMREILAEIQEGEFAREF
gi|83648555|ref|YP_436990.1|/1 K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGPEVINDSRAAMRNALKRITODGEYAKMF
gi|67159493|ref|ZP_00420011.1| K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGPEVINQSRQAMRNALKRITODGEYAKMF
gi|71065099|ref|YP_263826.1|/1 K-EVDLMEYEGCIADMNYSTSNNAEYGEYVTGPEVINQSRAMRNALKRITODGEYAKMF
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gi|74318007|ref|YP_315747.1|/1 K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGVKVINQSRAMKECLANQNGAYAKRF
gi|66044103|ref|YP_233944.1|/1 K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGPEVINAEESRQAMRNALKRITODGEYAKMF
gi|120553816|ref|YP_958167.1|/1 K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGPEVINQSRAMRNALKRITODGEYAKMF
gi|146306044|ref|YP_001186509.1| K-EVDLMEYEGCIANMNYSTSNNAEYGEYVTGPEVINAEESRKAMRNALKRITODGEYAKMF
gi|56552037|ref|YP_162876.1|/1 K-EVDLMEYEGCIANMRYSTNTAEYGDIVSGPRVINEESKKAMKAILDDTQSRFVSKF
gi|57240359|ref|ZP_00368308.1| K-EVDLLEYQCGCIADMRYSTNTAEYGDYITGPKIITKETKEAMKGVLDKIQNGSFAKDF
gi|6225553|sp|O32414|ILVC_RHOM K-EVDLMEYEGCIANMRYSTNTAEYGDYVTGSRIITEATKAEKRVLDITQSGRFVRDW
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gi|42781005|ref|NP_978252.1|/1 K-EVDLMEYEGCIANMRYSTNTAEYGDYVTGSRIITEATKAEKRVLDITQSGRFVRDW
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430 440 450 460 470 480

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gi|26991362|ref|NP_746787.1|/1 ISEGATNYP--SMTAKRRNNAAHGIE-IICEQTRSMMPWISANKIVDKTKN-----
gi|15599888|ref|NP_253382.1|/1 ITEGAANYP--SMTAYRRNNAAHPIE-QICEKTRSMMPWIAANKIVDKSKN-----
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gi|83648555|ref|YP_436990.1|/1 IAEGAHNYP--SMTAYRRNNAAHPIE-QVCEKTRSMMPWISANKIVDKSKN-----
gi|67159493|ref|ZP_00420011.1| ITEGAANYP--SMTAYRRNNAAHQIE-VVCEKTRSMMPWIAANKIVDKTKN-----
gi|71065099|ref|YP_263826.1|/1 ISEGATNYP--SMTARRRNNAAHQIE-ITGAKTRGMPWIGGKNIIDKDKN-----
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gi|15897495|ref|NP_342100.1|/1 VEEYGRGMP--TVVNGLSNVQNSLEE-KICNQTRDDEVQK-----GKPKS-----
gi|76801743|ref|YP_326751.1|/1 ISENQAGRP--SYKQLRAAEKNDHIE-AVGEDTRALFAW-----GDD-----
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gi|16079881|ref|NP_390707.1|/1 IVENQVNRP--RFNAINASENEHQIE-VVCRKTRSMMPFVKQGKKKEAVVVAQN-----
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gi|42781005|ref|NP_978252.1|/1 ILENQAGRP--TYNAMKKAEQNHQIE-KVCAETREMMSWIDAPKELVKK-----
gi|266346|sp|Q01292|ILV5_SPIOL VLAGRRFYEKEGLPAFFMGKIDQTRMKVCEKTRSRVP--AGD--LGPLYFTAGVYVAL

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gi|114319705|ref|YP_741388.1|/  ----
gi|83648555|ref|YP_436990.1|/1  ----
gi|67159493|ref|ZP_00420011.1|  ----
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gi|120553816|ref|YP_958167.1|/  ----
gi|146306044|ref|YP_001186509.  ----
gi|56552037|ref|YP_162876.1|/1  ----
gi|57240359|ref|ZP_00368308.1|  ----
gi|6225553|sp|O32414|ILVC_RHOM  ----
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gi|76801743|ref|YP_326751.1|/1  ----
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Figure 10

gi|70732562|ref|YP\_262325.1|: domain 1 of 1, from 1 to 338: score 926.8, E = 1e-270

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      M   kV+YDkD+dls   +i+GKkVA+IGYGSQGHAA+NL+D
gi|7073256   1   -M----KVFDKDCDLS-----IIQGKKVAIIIGYGSQGHAAQNLRD 37

      SGVdVvVGLRkGsaSwakAeaaGfKvktvaEAvaqADvVmiLlPDefQae
      SGVdV+VGLRkGsa++akAea+G+kV +va Ava+AD+VmiL+PDefQ++
gi|7073256   38  SGVDVTVGLRKGSAIVAKAEAHGLKVTDVAAAVAGADLVMILTPDEFQSQ 87

      vYeeeIepnLkpGatLaFAHGFNIHfqqIvPraFPkDiDViMVAPKpPGH
      +Y+++eIepn+k+GatLaF+HGF+IH++q+vPra   D+DViM+APK+PGH
gi|7073256   88  LYKNEIEPNIKKGATLAFSHGFALHYNQVVPRA---DLDVIMIAPKAPGH 134

      tVRreYvkGgVpALiAVyQDasGnAkdALsYAKgiGggRAGvIETTFk
      tVR+e+vkGg+P+LiA+yQDasGnAk++ALsYA+g+GggR+G+IETTFk
gi|7073256   135 TVRSEFVKGGGIPDLIAIYQDASGNAKNVALSYAAGVGGGRTGIETTFK 184

      eETETDLFGEQaVLCGGvteLVkaGFETLVEaGYaPEmAYFECLHELKLI
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      VDLmYEGGianMrySiSdTAeYGdyvtGprVideeskeaMkevLkdIQsG
      VDLmYEGGianM+ySiS++AeYG+yvtGp+VI++es++aM+++Lk+IQ+G
gi|7073256   235 VDLMEYEGGIANMNYSSISNNAEYGEYVTGPEVINAESRQAMRNALKRIQDG 284

      eFAkewilEnqaGyPketltalrrneaeHqIEWkVGekLRsmmpWiaanK
      e+Ak+++i+E+++GyP   ++ta rrn+a+H IE  +Ge+LRsmmpWI anK
gi|7073256   285 EYAKMFISEGATGYP--SMTAKRRNNAAHGIE-IIGEQLRSMPWIGANK 331

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      +vdk+kn
gi|7073256   332 IVDKAKN   338

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## KETOL-ACID REDUCTOISOMERASE USING NADH

**[0001]** This application claims the benefit of U.S. Provisional Applications, 61/015,346, filed Dec. 20, 2007, and 61/109,297, filed Oct. 29, 2008.

### FIELD OF THE INVENTION

**[0002]** The invention relates to protein evolution. Specifically, ketol-acid reductoisomerase enzymes have been evolved to use the cofactor NADH instead of NADPH.

### BACKGROUND OF THE INVENTION

**[0003]** Ketol-acid reductoisomerase enzymes are ubiquitous in nature and are involved in the production of valine and isoleucine, pathways that may affect the biological synthesis of isobutanol. Isobutanol is specifically produced from catabolism of L-valine as a by-product of yeast fermentation. It is a component of "fusel oil" that forms as a result of incomplete metabolism of amino acids by yeasts. After the amine group of L-valine is harvested as a nitrogen source, the resulting  $\alpha$ -keto acid is decarboxylated and reduced to isobutanol by enzymes of the Ehrlich pathway (Dickinson, et al., *J. Biol. Chem.* 273, 25752-25756, 1998).

**[0004]** Addition of exogenous L-valine to the fermentation increases the yield of isobutanol, as described by Dickinson et al., supra, wherein it is reported that a yield of isobutanol of 3 g/L is obtained by providing L-valine at a concentration of 20 g/L in the fermentation. In addition, production of n-propanol, isobutanol and isoamylalcohol has been shown by calcium alginate immobilized cells of *Zymomonas mobilis* (Oaxaca, et al., *Acta Biotechnol.*, 11, 523-532, 1991).

**[0005]** An increase in the yield of C3-C5 alcohols from carbohydrates was shown when amino acids leucine, isoleucine, and/or valine were added to the growth medium as the nitrogen source (WO 2005040392).

**[0006]** While methods described above indicate the potential of isobutanol production via biological means these methods are cost prohibitive for industrial scale isobutanol production. The biosynthesis of isobutanol directly from sugars would be economically viable and would represent an advance in the art. However, to date the only ketol-acid reductoisomerase (KARI) enzymes known are those that bind NADPH in its native form, reducing the energy efficiency of the pathway. A KARI that would bind NADH would be beneficial and enhance the productivity of the isobutanol biosynthetic pathway by capitalizing on the NADH produced by the existing glycolytic and other metabolic pathways in most commonly used microbial cells. The discovery of a KARI enzyme that can use NADH as a cofactor as opposed to NADPH would be an advance in the art.

**[0007]** The evolution of enzymes having specificity for the NADH cofactor as opposed to NADPH is known for some enzymes and is commonly referred to as "cofactor switching". See for example Eppink, et al. *J. Mol. Biol.*, (1999), 292, 87-96, describing the switching of the cofactor specificity of strictly NADPH-dependent p-Hydroxybenzoate hydroxylase (PHBH) from *Pseudomonas fluorescens* by site-directed mutagenesis; and Nakanishi, et al., *J. Biol. Chem.*, (1997), 272, 2218-2222, describing the use of site-directed mutagenesis on a mouse lung carbonyl reductase in which Thr-38 was replaced by Asp (T38D) resulting in an enzyme having a

200-fold increase in the  $K_m$  values for NADP(H) and a corresponding decrease of more than 7-fold in those for NAD(H). Co-factor switching has been applied to a variety of enzymes including monooxygenases, (Kamerbeek, et al., *Eur. J. Biochem.*, (2004), 271, 2107-2116); dehydrogenases; Nishiyama, et al., *J. Biol. Chem.*, (1993), 268, 4656-4660; Ferredoxin-NADP reductase, Martinez-Julvez, et al., *Biophys. Chem.*, (2005), 115, 219-224); and oxidoreductases (US2004/0248250).

**[0008]** Rane et al., (*Arch. Biochem. Biophys.*, (1997), 338, 83-89) discuss cofactor switching of a ketol acid reductoisomerase isolated from *E. coli* by targeting four residues in the enzyme for mutagenesis, (R68, K69, K75, and R76.); however the effectiveness of this method is in doubt.

**[0009]** Although the above cited methods suggest that it is generally possible to switch the cofactor specificity between NADH and NADPH, the methods are enzyme specific and the outcomes unpredictable. The development of a ketol-acid reductoisomerase having a high specificity for NADH as opposed to NADPH would greatly enhance its effectiveness in the isobutanol biosynthetic pathway, however, no such KARI enzyme has been reported.

**[0010]** Applicants have solved the stated problem by identifying a number of mutant ketol-acid reductoisomerase enzymes that have a preference for binding NADH as opposed to NADPH.

### SUMMARY OF THE INVENTION

**[0011]** The invention relates to a method for the evolution of ketol-acid reductoisomerase (KARI) enzymes from binding the cofactor NADPH to binding NADH. The method involves mutagenesis of certain specific residues in them KARI enzyme to produce the co-factor switching.

**[0012]** Accordingly the invention provides a mutant ketol-acid reductoisomerase enzyme comprising the amino acid sequence as set forth in SEQ ID NO: 29.

**[0013]** Alternatively the invention provides a mutant ketol-acid reductoisomerase enzyme having the amino acid sequence selected from the group consisting of SEQ ID NO: 19, 24, 25, 26, 27, 28, 67, 68, 69, and 70.

**[0014]** In a preferred embodiment a mutant ketol-acid reductoisomerase enzyme is provided as set forth in SEQ ID NO:17 comprising at least one mutation at a residue selected from the group consisting of 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, 165, and 170.

**[0015]** In a specific embodiment the invention provides a mutant ketol-acid reductoisomerase enzyme as set forth in SEQ ID NO:17 wherein:

**[0016]** a) the residue at position 47 has an amino acid substitution selected from the group consisting of A, C, D, F, G, I, L, N, P, and Y;

**[0017]** b) the residue at position 50 has an amino acid substitution selected from the group consisting of A, C, D, E, F, G, M, N, V, W;

**[0018]** c) the residue at position 52 has an amino acid substitution selected from the group consisting of A, C, D, G, H, N, S;

**[0019]** d) the residue at position 53 has an amino acid substitution selected from the group consisting of A, H, I, W;

**[0020]** e) the residue at position 156 has an amino acid substitution of V;

**[0021]** f) the residue at position 165 has an amino acid substitution of M;

- [0022] g) the residue at position 61 has an amino acid substitution of F;
- [0023] h) the residue at position 170 has an amino acid substitution of A;
- [0024] i) the residue at position 24 has an amino acid substitution of F;
- [0025] j) the residue at position 33 has an amino acid substitution of L;
- [0026] k) the residue at position 80 has an amino acid substitution of I; and
- [0027] l) the residue at position 115 has an amino acid substitution of L.
- [0028] In another embodiment the invention provides a method for the evolution of a NADPH binding ketol-acid reductoisomerase enzyme to an NADH using form comprising:
- [0029] a) providing a ketol-acid reductoisomerase enzyme which uses NADPH having a specific native amino acid sequence;
- [0030] b) identifying the cofactor switching residues in the enzyme of a) based on the amino acid sequence of the *Pseudomonas fluorescens* ketol-acid reductoisomerase enzyme as set for the in SEQ ID NO:17 wherein the cofactor switching residues are at positions selected from the group consisting of; 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, 165, and 170;
- [0031] c) creating mutations in at least one of the cofactor switching residues of b) to create a mutant enzyme wherein said mutant enzyme binds NADH.
- [0032] In an alternate embodiment the invention provides a method for the production of isobutanol comprising:
- [0033] a) providing a recombinant microbial host cell comprising the following genetic constructs:
- [0034] i) at least one genetic construct encoding an acetolactate synthase enzyme for the conversion of pyruvate to acetolactate;
- [0035] ii) at least one genetic construct encoding a mutant ketol-acid reductoisomerase enzyme of the invention;
- [0036] iii) at least one genetic construct encoding an acetohydroxy acid dehydratase for the conversion of 2,3-dihydroxyisovalerate to  $\alpha$ -ketoisovalerate, (pathway step c);
- [0037] iv) at least one genetic construct encoding a branched-chain keto acid decarboxylase, of the conversion of  $\alpha$ -ketoisovalerate to isobutyraldehyde, (pathway step d);
- [0038] v) at least one genetic construct encoding a branched-chain alcohol dehydrogenase for the conversion of isobutyraldehyde to isobutanol (pathway step e); and
- [0039] b) growing the host cell of (a) under conditions where iso-butanol is produced.

#### BRIEF DESCRIPTION OF THE FIGURES SEQUENCE DESCRIPTIONS

- [0040] The invention can be more fully understood from the following detailed description, the Figures, and the accompanying sequence descriptions, which form part of this application.
- [0041] FIG. 1—Shows four different isobutanol biosynthetic pathways. The steps labeled “a”, “b”, “c”, “d”, “e”, “f”, “g”, “h”, “i”, “j” and “k” represent the substrate to product conversions described below.

[0042] FIG. 2—Multiple sequence alignment (MSA) of KARI enzymes from different recourses. (a) MSA among three NADPH-requiring KARI enzymes; (b) MSA among PF5-KARI and other KARI enzymes, with promiscuous nucleotide specificity, where, MMC5—is from *Methanococcus maripaludis* C5; MMS2—is from *Methanococcus maripaludis* S2; MNSB—is from *Methanococcus vannielii* SB; ilv5—is from *Saccharomyces cerevisiae* ilv5; KARI-D1—is from *Sulfolobus solfataricus* P2 ilvC; KARI-D2—is from *Pyrobaculum aerophilum* P2ilvC; and KARI S1—is from *Ralstonia solanacearum* GMI1000 ivIC.

[0043] FIG. 3—Interaction of phosphate binding loop with NADPH based on homology modeling.

[0044] FIG. 4: KARI activities of top performers from the library C using cofactors NADH versus NADPH. Activity and standard deviation were derived from triple experiments. The mutation information is as follows: C3A7=R47Y/S50A/T52D/V53W; C3A10=R47Y/S50A/T52G/V53W; C3B11=R47F/S50A/T52D/V53W; C3C8=R47G/S50M/T52D/V53W; and C4D12=R47C/S50MT52D/V53W

[0045] FIG. 5—(a) KARI activities of top performers from libraries E, F and G using cofactors NADH versus NADPH. (b) KARI activities of positive control versus wild type Pf5-ilvC using cofactors NADH. Activity and standard deviation were derived from at least three parallel experiments. “Wt” represents the wild type of Pf5-ilvC and “Neg” means negative control.

Experiments for NADH and NADPH reactions in (a) were 30 minutes; in (b) were 10 minutes.

[0046] FIG. 6—Activities of top performers from library H using cofactors NADH versus NADPH. Activity and standard deviation were derived from triple experiments. Mutation information is as follows: 24F9=R47P/S50G/T52D; 68F10=R47P/T52S; 83G10=R47P/S50D/T52S; 39G4=R47P/S50C/T52D; 91A9=R47P/S50CT52D; and C3B11=R47F/S50A/T52D/V53W

[0047] FIG. 7—Thermostability of PF5-ilvC. The remaining activity of the enzyme after heating at certain temperatures for 10 min was the average number of triple experiments and normalized to the activity measured at room temperature.

[0048] FIG. 8—Multiple sequence alignment among 5 naturally existing KARI molecules. The positions bolded and grey highlighted were identified by error prone PCR and the positions only grey highlighted were targeted for mutagenesis.

[0049] FIG. 9—Alignment of the twenty-four functionally verified KARI sequences. The GxGXX(G/A) motif involved in the binding of NAD(P)H is indicated below the alignment.

[0050] FIG. 10—An example of the alignment of *Pseudomonas fluorescens* Pf-5 KARI to the profile HMM of KARI. The eleven positions that are responsible for co-factor switching are bolded and shaded in grey.

[0051] Table 9—is a table of the Profile HMM of the KARI enzymes described in Example 5. The eleven positions in the profile HMM representing the columns in the alignment which correspond to the eleven cofactor switching positions in *Pseudomonas fluorescens* Pf-5 KARI are identified as positions 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, and 170. The lines corresponding to these positions in the model file are highlighted in yellow. Table 9 is submitted herewith electronically and is incorporated herein by reference.

[0052] The following sequences conform with 37 C.F.R. 1.821-1.825 (“Requirements for Patent Applications Con-

taining Nucleotide Sequences and/or Amino Acid Sequence Disclosures—the Sequence Rules”) and are consistent with the World Intellectual Property Organization (WIPO) Standard ST.25 (1998) and the sequence listing requirements of

the EPO and PCT (Rules 5.2 and 49.5(a-bis), and Section 208 and Annex C of the Administrative Instructions). The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

TABLE 1

OLIGONUCLEOTIDE PRIMERS USED IN THIS INVENTION		
SEQUENCE ID No.	SEQUENCE	Description
1	TGATGAACATCTTCGCGTATTCGCCGTCCT	Reverse Primer for pBAD vector
2	GCGTAGACGTGACTGTTGGCCTGNNTAAAGGCNN GGCTNNCTGGGCCAAGGCT GAAGCCACGGCTTG	Forward primer library C
3	GCGTAGACGTGACTGTTGGCCTGNNTAAAGGCTCG GCTACCGTTGCCAAGGCTGAAGCCACGGCTTG	Forward primer for library E
4	GCGTAGACGTGACTGTTGGCCTGCGTAAAGGCNNNT GCTACCGTTGCCAAGGCTGAAGCCACGGCTTG	Forward primer for library F
5	GCGTAGACGTGACTGTTGGCCTGCGTAAAGGCTCG GCTNNTGTTGCCAAGGCTGAAGCCACGGCTTG	Forward primer for library G
6	GCGTAGACGTGACTGTTGGCCTGNNTAAAGGCNNNT GCTNNTGTTGCCAAGGCTGAAGCCACGGCTTG	Forward primer for library H
7	AAGATTAGCGGATCCTACCT	Sequencing primer (forward)
8	AACAGCCAAGCTTTTAGTTC	Sequencing primer (reverse)
20	CTCTCTACTGTTTCTCCATACCCG	pBAD_266-021308f
21	CAAGCCGTGGGCTTCAGCCTTGCKNN	PF5_53Mt022908r
22	CGGTTTCAGTCTCGTCCTGAAG	pBAD_866-021308
49	GCTCAAGCANNKAACCTGAAGG	pBAD-405- C33_090808f
50	CCTTCAGGTTKNNTGCTTGAGC	pBAD-427- C33_090808r
51	GTAGACGTGNNKGTGGCCTG	pBAD-435- T43_090808f
52	CAGGCCAACKNNCAGTCTAC	pBAD-456- T43_090808r
53	CTGAAGCCNNKGGCNNKAAAGTGAC	pBAD-484- H59L61_090808f
54	GTCACTTTKNNGCCKNNGCCTTCAG	pBAD-509- H59L61_090808r
55	GCAGCCGTTNNKGGTGCCGACT	pBAD-519- A71_090808f
56	AGTCGGCACCKNNAACGGCTGC	pBAD-541- A71_090808r
57	CATGATCCTGNNKCCGGAGGAG	pBAD-545- T80_090808f
58	CTCGTCCGGKNNCAGGATCATG	pBAD-567- T80_090808r
59	CAAGAAGGGCNNKACTCTGGCCT	pBAD-608- A101_090808f
60	AGGCCAGAGTKNNGCCCTTCTTG	pBAD-631- A101_090808r

TABLE 1-continued

OLIGONUCLEOTIDE PRIMERS USED IN THIS INVENTION		
SEQUENCE ID No.	SEQUENCE	Description
61	GTTGTGCCTNNKCCGACCTCG	pBAD-663- R119_090808f
62	CGAGGTCGGCKNNAGGCACAAC	pBAD-685- R119_090808r

[0053] Additional sequences used in the application are listed below. The abbreviated gene names in bracket are used in this disclosure.

SEQ ID NO: 9-*Methanococcus maripaludis* C5-ilvC (MMC5)—GenBank Accession Number NC\_009135.1 Region: 901034.902026

SEQ ID NO: 10 is the *Methanococcus maripaludis* S2-ilvC (MMS2)—GenBank Accession Number NC\_005791.1 Region: 645729.646721

SEQ ID NO: 11 is the *Methanococcus vannielii* SB-ilv5 (MVS5)—GenBank Accession Number NZ\_AAAX01000002.1 Region: 302214.303206

SEQ ID NO: 12 is the *Saccharomyces cerevisiae* ilv5 (ilv5)—GenBank Accession Number NC\_001144.4 Region: 838065.839252

SEQ ID NO: 13 is the *Sulfolobus solfataricus* P2 ilvC (KARI-D1)—GenBank Accession Number NC\_002754.1 Region: 506253.507260

SEQ ID NO: 14 is the *Pyrobaculum aerophilum* str. IM2 ilvC (KARI-D2)—GenBank Accession Number NC\_003364.1 Region: 1976281.1977267

SEQ ID NO: 15 is the *Ralstonia solanacearum* GMI1000 ilvC (KARI-S1)—GenBank Accession Number NC\_003295.1 Region: 2248264.2249280

SEQ ID NO: 16 is the *Pseudomonas aeruginosa* PAO1 ilvC—GenBank Accession Number NC\_002516 Region: 5272455.5273471

SEQ ID NO: 17 is the *Pseudomonas fluorescens* PF5 ilvC—GenBank Accession Number NC\_004129 Region: 6017379.6018395

SEQ ID NO: 18 is the *Spinacia oleracea* ilvC (Spinach-KARI)—GenBank Accession Number NC\_002516 Region: 1.2050.

SEQ ID NO: 19 is the amino acid sequence of the mutant (Y24F/R47Y/S50A/T52D/V53A/L61F/G170A) of the ilvC native protein of *Pseudomonas fluorescens*.

SEQ ID NO: 23 is the DNA SEQ of the mutant (Y24F/R47Y/S50A/T52D/V53A/L61F/G170A) of the ilvC native protein of *Pseudomonas fluorescens*.

SEQ ID NO: 24 is the amino acid SEQ of the mutant ZB1 (Y24F/R47Y/S50A/T52D/V53A/L61F/A156V)

SEQ ID NO: 25 is the amino acid SEQ of the mutant ZF3 (Y24F/C33L/R47Y/S50A/T52D/V53A/L61F)

SEQ ID NO: 26 is the amino acid SEQ of the mutant ZF2 (Y24F/C33L/R47Y/S50A/T52D/V53A/L61F/A156V)

SEQ ID NO: 27 is the amino acid SEQ of the mutant ZB3 (Y24F/C33L/R47Y/S50A/T52D/V53A/L61F/G170A)

SEQ ID NO: 28 is the amino acid SEQ of the mutant Z4B8 (C33L/R47Y/S50A/T52D/V53A/L61F/T801/A156V/G170A)

SEQ ID NO: 29 is a consensus amino acid sequence comprising all experimentally verified KARI point mutations as based on SEQ ID NO:17.

SEQ ID NO: 30 is the amino acid sequence for KARI from *Natronomonas pharaonis* DSM 2160

SEQ ID NO: 31 is the amino acid sequence for KARI from *Bacillus subtilis* subsp. *subtilis* str. 168

SEQ ID NO: 32 is the amino acid sequence for KARI from *Corynebacterium glutamicum* ATCC13032

SEQ ID NO: 33 is the amino acid sequence for KARI from *Phaeosporium molischianum*

SEQ ID NO: 34 is the amino acid sequence for KARI from *Zymomonas mobilis* subsp. *mobilis* ZM4

SEQ ID NO: 35 is the amino acid sequence for KARI *Alkalilimnicola ehrlichei* MLHE-1

SEQ ID NO: 36 is the amino acid sequence for KARI from *Campylobacter lari* RM2100

SEQ ID NO: 37 is the amino acid sequence for KARI from *Marinobacter aquaeolei* VT8

SEQ ID NO: 38 is the amino acid sequence for KARI *Psychrobacter arcticus* 273-4

SEQ ID NO: 39 is the amino acid sequence for KARI from *Hahella chejuensis* KCTC2396

SEQ ID NO: 40 is the amino acid sequence for KARI from *Thiobacillus denitrificans* ATCC25259

SEQ ID NO: 41 is the amino acid sequence for KARI from *Azotobacter vinelandii* AVOP

SEQ ID NO: 42 is the amino acid sequence for KARI from *Pseudomonas syringae* pv. *syringae* B728a

SEQ ID NO: 43 is the amino acid sequence for KARI from *Pseudomonas syringae* pv. *tomato* str. DC3000

SEQ ID NO: 44 is the amino acid sequence for KARI from *Pseudomonas putida* KT2440

SEQ ID NO: 45 is the amino acid sequence for KARI from *Pseudomonas entomophila* L48

SEQ ID NO: 46 is the amino acid sequence for KARI from *Pseudomonas mendocina* ymp

SEQ ID NO: 47 is the amino acid sequence for KARI from *Bacillus cereus* ATCC10987 NP\_977840.1

SEQ ID NO: 48 is the amino acid sequence for KARI from *Bacillus cereus* ATCC10987 NP\_978252.1

SEQ ID NO: 63 is the amino acid sequence for KARI from *Escherichia coli*-GenBank Accession Number P05793

SEQ ID NO: 64 is the amino acid sequence for KARI from Marine Gamma Proteobacterium HTCC2207-GenBank Accession Number ZP\_01224863.1

SEQ ID NO: 65 is the amino acid sequence for KARI from *Desulfuromonas acetoxidans*—GenBank Accession Number ZP\_01313517.1



SEQ ID NO: 66 is the amino acid sequence for KARI from *Pisum sativum* (Pea)—GenBank Accession Number 082043  
SEQ ID NO: 67 is the amino acid sequence for the mutant 3361 G8 (C33L/R47Y/S50A/T52D/V53A/L61F/T801)

SEQ ID NO: 68 is the amino acid sequence for the mutant 2H10 (Y24F/C33L/R47Y/S50A/T52D/V531/L61F/T801/A156V)

SEQ ID NO: 69 is the amino acid sequence for the mutant 1D2 (Y24F/R47Y/S50A/T52D/V53A/L61F/T801/A156V)

SEQ ID NO: 70 is the amino acid sequence for the mutant 3F12 (Y24F/C33L/R47Y/S50A/T52D/V53A/L61F/T801/A156V).

#### DETAILED DESCRIPTION OF THE INVENTION

**[0054]** The present invention relates to the generation of mutated KARI enzymes to use NADH as opposed to NADPH. These co-factor switched enzymes function more effectively in microbial systems designed to produce isobutanol. Isobutanol is an important industrial commodity chemical with a variety of applications, where its potential as a fuel or fuel additive is particularly significant. Although only a four-carbon alcohol, butanol has the energy content similar to that of gasoline and can be blended with any fossil fuel. Isobutanol is favored as a fuel or fuel additive as it yields only CO<sub>2</sub> and little or no SO<sub>x</sub> or NO<sub>x</sub> when burned in the standard internal combustion engine. Additionally butanol is less corrosive than ethanol, the most preferred fuel additive to date.

**[0055]** The following definitions and abbreviations are to be used for the interpretation of the claims and the specification.

**[0056]** The term “invention” or “present invention” as used herein is meant to apply generally to all embodiments of the invention as described in the claims as presented or as later amended and supplemented, or in the specification.

**[0057]** The term “isobutanol biosynthetic pathway” refers to the enzymatic pathway to produce isobutanol. Preferred isobutanol biosynthetic pathways are illustrated in FIG. 1 and described herein.

**[0058]** The term “NADPH consumption assay” refers to an enzyme assay for the determination of the specific activity of the KARI enzyme, involving measuring the disappearance of the KARI cofactor, NADPH, from the enzyme reaction.

**[0059]** “KARI” is the abbreviation for the enzyme Ketol-acid reductoisomerase.

**[0060]** The term “close proximity” when referring to the position of various amino acid residues of a KARI enzyme with respect to the adenosyl 2'-phosphate of NADPH means amino acids in the three-dimensional model for the structure of the enzyme that are within about 4.5 Å of the phosphorus atom of the adenosyl 2'-phosphate of NADPH bound to the enzyme.

**[0061]** The term “Ketol-acid reductoisomerase” (abbreviated “KARI”), and “Acetohydroxy acid isomeroreductase” will be used interchangeably and refer to the enzyme having the EC number, EC 1.1.1.86 (*Enzyme Nomenclature* 1992, Academic Press, San Diego). Ketol-acid reductoisomerase catalyzes the reaction of (S)-acetolactate to 2,3-dihydroxyisovalerate, as more fully described below. These enzymes are available from a number of sources, including, but not limited to *E. coli* GenBank Accession Number NC-000913 REGION: 3955993.3957468, *Vibrio cholerae* GenBank Accession Number NC-002505 REGION: 157441.158925, *Pseudomonas aeruginosa*, GenBank Accession Number

NC-002516, (SEQ ID NO: 16) REGION: 5272455.5273471, and *Pseudomonas fluorescens* GenBank Accession Number NC-004129 (SEQ ID NO: 17) REGION: 6017379.6018395. As used herein the term “Class I ketol-acid reductoisomerase enzyme” means the short form that typically has between 330 and 340 amino acid residues, and is distinct from the long form, called class II, that typically has approximately 490 residues.

**[0062]** The term “acetolactate synthase” refers to an enzyme that catalyzes the conversion of pyruvate to acetolactate and CO<sub>2</sub>. Acetolactate has two stereoisomers ((R)— and (S)—); the enzyme prefers the (S)— isomer, which is made by biological systems. Preferred acetolactate synthases are known by the EC number 2.2.1.6.9 (*Enzyme Nomenclature* 1992, Academic Press, San Diego). These enzymes are available from a number of sources, including, but not limited to, *Bacillus subtilis* (GenBank Nos: CAB15618, Z99122, NCBI (National Center for Biotechnology Information) amino acid sequence, NCBI nucleotide sequence, respectively), *Klebsiella pneumoniae* (GenBank Nos: AAA25079 (SEQ ID NO:2), M73842 (SEQ ID NO:1)), and *Lactococcus lactis* (GenBank Nos: AAA25161, L16975).

**[0063]** The term “acetohydroxy acid dehydratase” refers to an enzyme that catalyzes the conversion of 2,3-dihydroxyisovalerate to  $\alpha$ -ketoisovalerate. Preferred acetohydroxy acid dehydratases are known by the EC number 4.2.1.9. These enzymes are available from a vast array of microorganisms, including, but not limited to, *E. coli* (GenBank Nos: YP\_026248, NC\_000913, *S. cerevisiae* (GenBank Nos: NP\_012550, NC\_001142), *M. maripaludis* (GenBank Nos: CAF29874, BX957219), and *B. subtilis* (GenBank Nos: CAB14105, Z99115).

**[0064]** The term “branched-chain  $\alpha$ -keto acid decarboxylase” refers to an enzyme that catalyzes the conversion of  $\alpha$ -ketoisovalerate to isobutyraldehyde and CO<sub>2</sub>. Preferred branched-chain  $\alpha$ -keto acid decarboxylases are known by the EC number 4.1.1.72 and are available from a number of sources, including, but not limited to, *Lactococcus lactis* (GenBank Nos: AAS49166, AY548760; CAG34226, AJ746364, *Salmonella typhimurium* (GenBank Nos: NP-461346, NC-003197), and *Clostridium acetobutylicum* (GenBank Nos: NP-149189, NC-001988).

**[0065]** The term “branched-chain alcohol dehydrogenase” refers to an enzyme that catalyzes the conversion of isobutyraldehyde to isobutanol. Preferred branched-chain alcohol dehydrogenases are known by the EC number 1.1.1.265, but may also be classified under other alcohol dehydrogenases (specifically, EC 1.1.1.1 or 1.1.1.2). These enzymes utilize NADH (reduced nicotinamide adenine dinucleotide) and/or NADPH as electron donor and are available from a number of sources, including, but not limited to, *S. cerevisiae* (GenBank Nos: NP-010656, NC-001136; NP-014051, NC-001145), *E. coli* (GenBank Nos: NP-417-484, and *C. acetobutylicum* (GenBank Nos: NP-349892, NC\_003030).

**[0066]** The term “branched-chain keto acid dehydrogenase” refers to an enzyme that catalyzes the conversion of  $\alpha$ -ketoisovalerate to isobutyryl-CoA (isobutyryl-cofactor A), using NAD<sup>+</sup> (nicotinamide adenine dinucleotide) as electron acceptor. Preferred branched-chain keto acid dehydrogenases are known by the EC number 1.2.4.4. These branched-chain keto acid dehydrogenases comprise four subunits, and sequences from all subunits are available from a vast array of microorganisms, including, but not limited to, *B. subtilis* (GenBank Nos: CAB14336, Z99116; CAB14335, Z99116;

CAB14334, Z99116; and CAB14337, Z99116) and *Pseudomonas putida* (GenBank Nos: AAA65614, M57613; AAA65615, M57613; AAA65617, M57613; and AAA65618, M57613).

[0067] The terms “ $k_{cat}$ ” and “ $K_m$ ” are known to those skilled in the art and are described in Enzyme Structure and Mechanism, 2<sup>nd</sup> ed. (Ferst; W.H. Freeman: NY, 1985; pp 98-120). The term “ $k_{cat}$ ”, often called the “turnover number”, is defined as the maximum number of substrate molecules converted to products per active site per unit time, or the number of times the enzyme turns over per unit time.  $k_{cat} = V_{max}/[E]$ , where [E] is the enzyme concentration (Ferst, supra). The terms “total turnover” and “total turnover number” are used herein to refer to the amount of product formed by the reaction of a KARI enzyme with substrate.

[0068] The term “catalytic efficiency” is defined as the  $k_{cat}/K_M$  of an enzyme. Catalytic efficiency is used to quantify the specificity of an enzyme for a substrate.

[0069] The term “isolated nucleic acid molecule”, “isolated nucleic acid fragment” and “genetic construct” will be used interchangeably and will mean a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

[0070] The term “amino acid” refers to the basic chemical structural unit of a protein or polypeptide. The following abbreviations are used herein to identify specific amino acids:

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0071] The term “Gene” refers to a nucleic acid fragment that is capable of being expressed as a specific protein, optionally including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers to any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. “Endogenous

gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

[0072] As used herein the term “Coding sequence” refers to a DNA sequence that codes for a specific amino acid sequence. “Suitable regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing site, effector binding site and stem-loop structure.

[0073] The term “Promoter” refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

[0074] 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.

[0075] The term “expression”, as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide.

[0076] As used herein the term “transformation” refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” or “recombinant” or “transformed” organisms.

[0077] The terms “plasmid”, “vector” and “cassette” refer to an extra chromosomal element often carrying genes which are not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA fragments. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a

unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell. "Transformation cassette" refers to a specific vector containing a foreign gene and having elements in addition to the foreign gene that facilitates transformation of a particular host cell. "Expression cassette" refers to a specific vector containing a foreign gene and having elements in addition to the foreign gene that allow for enhanced expression of that gene in a foreign host.

**[0078]** 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.

**[0079]** 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.

**[0080]** Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook et al. (Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) (hereinafter "Maniatis"); and by Silhavy et al. (*Experiments with Gene Fusions*, Cold Spring Harbor Laboratory Press Cold Spring Harbor, N.Y., 1984); and by Ausubel, F. M. et al., (*Current Protocols in Molecular Biology*, published by Greene Publishing Assoc. and Wiley-Interscience, 1987).

**[0081]** The present invention addresses a need that arises in the microbial production of isobutanol where the ketol-acid reductoisomerase enzyme performs a vital role. Wild type ketol-acid reductoisomerase enzymes typically use NADPH as their cofactor. However, in the formation of isobutanol an excess of NADH is produced by ancillary metabolic pathways. The invention provides mutant Class I KARI enzymes that have been evolved to utilize NADH as a cofactor, overcoming the cofactor problem and increasing the efficiency of the isobutanol biosynthetic pathway.

**[0082]** Production of isobutanol utilizes the glycolysis pathway present in the host organism. During the production of two molecules of pyruvate from glucose during glycolysis, there is net production of two molecules of NADH from NAD<sup>+</sup> by the glyceraldehyde-3-phosphate dehydrogenase reaction. During the further production of one molecule of isobutanol from two molecules of pyruvate, there is net consumption of one molecule of NADPH, by the KARI reaction, and one molecule of NADH by the isobutanol dehydrogenase reaction. The overall reaction of glucose to isobutanol thus leads to net production of one molecule of NADH and net consumption of one molecule of NADPH. The interconversion of NADH with NADPH is generally slow and inefficient; thus, the NADPH consumed is generated by metabolism (for example, by the pentose phosphate pathway) consuming substrate in the process. Meanwhile, the cell strives to maintain homeostasis in the NAD<sup>+</sup>/NADH ratio, leading to the excess

NADH produced in isobutanol production being consumed in wasteful reduction of other metabolic intermediates; e.g., by the production of lactate from pyruvate. Thus, the imbalance between NADH produced and NADPH consumed by the isobutanol pathway leads to a reduction in the molar yield of isobutanol produced from glucose in two ways: 1) unnecessary operation of metabolism to produce NADPH, and 2) wasteful reaction of metabolic intermediates to maintain NAD<sup>+</sup>/NADH homeostasis. The solution to this problem is to invent a KARI that is specific for NADH as its cofactor, so that both molecules of NADH produced in glycolysis are consumed in the synthesis of isobutanol from pyruvate.

#### Keto Acid Reductoisomerase (KARI) Enzymes

**[0083]** Acetohydroxy acid isomeroreductase or Ketol-acid reductoisomerase (KARI; EC 1.1.1.86) catalyzes two steps in the biosynthesis of branched-chain amino acids and is a key enzyme in their biosynthesis. KARI is found in a variety of organisms and amino acid sequence comparisons across species have revealed that there are 2 types of this enzyme: a short form (class I) found in fungi and most bacteria, and a long form (class II) typical of plants.

**[0084]** Class I KARIs typically have between 330-340 amino acid residues. The long form KARI enzymes have about 490 amino acid residues. However, some bacteria such as *Escherichia Coli* possess a long form, where the amino acid sequence differs appreciably from that found in plants. KARI is encoded by the *ilvC* gene and is an essential enzyme for growth of *E. Coli* and other bacteria in a minimal medium. Typically KARI uses NADPH as cofactor and requires a divalent cation such as Mg<sup>++</sup> for its activity. In addition to utilizing acetolactate in the valine pathway, KARI also converts acetohydroxybutanoate to dihydroxymethylpentanoate in the isoleucine production pathway.

**[0085]** Class II KARIs generally consist of a 225-residue N-terminal domain and a 287-residue C-terminal domain. The N-terminal domain, which contains the NADPH-binding site, has an  $\alpha/\beta$  structure and resembles domains found in other pyridine nucleotide-dependent oxidoreductases. The C-terminal domain consists almost entirely of  $\alpha$ -helices and is of a previously unknown topology.

**[0086]** The crystal structure of the *E. Coli* KARI enzyme at 2.6 Å resolution has been solved (Tyagi, et al., *Protein Science*, 14, 3089-3100, 2005). This enzyme consists of two domains, one with mixed  $\alpha/\beta$  structure which is similar to that found in other pyridine nucleotide-dependent dehydrogenases. The second domain is mainly  $\alpha$ -helical and shows strong evidence of internal duplication. Comparison of the active sites of KARI of *E. Coli*, *Pseudomonas aeruginosa*, and spinach showed that most residues in the active site of the enzyme occupy conserved positions. While the *E. Coli* KARI was crystallized as a tetramer, which is probably the likely biologically active unit, the *P. aeruginosa* KARI (Ahn, et al., *J. Mol. Biol.*, 328, 505-515, 2003) formed a dodecamer, and the enzyme from spinach formed a dimer. Known KARIs are slow enzymes with a reported turnover number ( $k_{cat}$ ) of 2 s<sup>-1</sup> (Aulabaugh et al.; *Biochemistry*, 29, 2824-2830, 1990) or 0.12 s<sup>-1</sup> (Rane et al., *Arch. Biochem. Biophys.* 338, 83-89, 1997) for acetolactate. Studies have shown that genetic control of isoleucine-valine biosynthesis in *E. coli* is different than that in *Ps. aeruginosa* (Marinus, et al., *Genetics*, 63, 547-56, 1969).

#### Identification of Amino Acid Target Sites for Cofactor Switching

**[0087]** It was reported that phosphate p2' oxygen atoms of NADPH form hydrogen bonds with side chains of Arg162,

Ser165 and Ser167 of spinach KARI (Biou V. et al. *The EMBO Journal*, 16: 3405-3415, 1997). Multiple sequence alignments were performed, using vector NTI (Invitrogen Corp. Carlsbad, Calif.), with KARI enzymes from spinach, *Pseudomonas aeruginosa* (PAO-KARI) and *Pseudomonas fluorescens* (PF5-KARI). The NADPH binding sites are shown in FIG. 2a. The amino acids, arginine, threonine and serine appear to play similar roles in forming hydrogen bonds with phosphate p2' oxygen atoms of NADPH in KARI enzymes. Studies by Ahn et al (J. Mol. Biol., 328: 505-515, 2003) had identified three NADPH phosphate binding sites (Arg47, Ser50 and Thr52) for *Pseudomonas aeruginosa* (PAO-KARI) following comparing its structure with that of the spinach KARI. Hypothesizing that these three NADPH phosphate binding sites of the three KARI enzymes used in the disclosure were conserved, Arg47, Ser50 and Thr52 of PF5-KARI were targeted as the phosphate binding sites for this enzyme. This hypothesis was further confirmed through homology modeling.

[0088] Multiple sequence alignment among PF5-ilvC and several other KARI enzymes with promiscuous nucleotide specificity was also performed. As shown in FIG. 2b, the amino acids of glycine (G50) and tryptophan (W53), in other KARI enzymes in FIG. 2b, always appear together as a pair in the sequences of those enzymes. It was therefore assumed that the tryptophan 53 bulky residue was important in determining nucleotide specificity by reducing the size of nucleotide binding pocket to favor the smaller nucleotide, NADH. Position 53 of PF5-ilvC was therefore chosen as a target for mutagenesis.

[0089] Several site-saturation gene libraries were prepared containing genes encoding KARI enzymes by commercially available kits for the generation of mutants. Clones from each library were screened for improved KARI activity using the NADH consumption assay described herein. Screening resulted in the identification of a number of genes having mutations that can be correlated to KARI activity. The location of the mutations were identified using the amino acid sequence the *Pseudomonas fluorescens* PF5 ilvC protein (SEQ ID NO:17). Mutants having improved KARI activity were those which had mutations at the following positions: 47, 50, 52 and 53. More specifically desirable mutations included the following substitutions:

[0090] a) the residue at position 47 has an amino acid substitution selected from the group consisting of A, C, D, F, G, I, L, N, P, and Y;

[0091] b) the residue at position 50 has an amino acid substitution selected from the group consisting of A, C, D, E, F, G, M, N, V, W;

[0092] c) the residue at position 52 has an amino acid substitution selected from the group consisting of A, C, D, G, H, N, S;

[0093] d) the residue at position 53 has an amino acid substitution selected from the group consisting of A, H, I, W;

[0094] In another embodiment, additional mutagenesis, using error prone PCR, performed on the mutants listed above identified suitable mutation positions as: 156, 165, 61, 170, 115 and 24. More specifically the desirable mutants with lower  $K_m$  for NADH contained the following substitutions:

[0095] e) the residue at position 156 has an amino acid substitution of V;

[0096] f) the residue at position 165 has an amino acid substitution of M;

[0097] g) the residue at position 61 has an amino acid substitution of F;

[0098] h) the residue at position 170 has an amino acid substitution of A;

[0099] i) the residue at position 24 has an amino acid substitution of F; and

[0100] j) the residue at position 115 has an amino acid substitution of L.

[0101] In further work, multiple sequence alignment of *Pseudomonas fluorescens* PF5-ilvC and *Bacillus cereus* ilvC1 and ilvC2 and spinach KARI was performed which allowed identification of positions 24, 33, 47, 50, 52, 53, 61, 80, 156 and 170 for further mutagenesis. More specifically mutants with much lower  $K_m$  for NADH were obtained. These mutations are also based on the *Pseudomonas fluorescens*, KARI enzyme (SEQ ID NO:17) as a reference sequence wherein the reference sequence comprises at least one amino acid substitution selected from the group consisting of:

[0102] k) the residue at position 24 has an amino acid substitution of phenylalanine;

[0103] l) the residue at position 50 has an amino acid substitution of alanine;

[0104] m) the residue at position 52 has an amino acid substitution of aspartic acid;

[0105] n) the residue at position 53 has an amino acid substitution of alanine;

[0106] o) the residue at position 61 has an amino acid substitution of phenylalanine;

[0107] p) the residue at position 156 has an amino acid substitution of valine;

[0108] q) the residue at position 33 has an amino acid substitution of leucine;

[0109] r) the residue at position 47 has an amino acid substitution of tyrosine;

[0110] s) the residue at position 80 has an amino acid substitution of isoleucine; and

[0111] t) the residue at position 170 has an amino acid substitution of alanine.

[0112] The present invention includes a mutant polypeptide having KARI activity, said polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 24, 25, 26, 27 and 28.

[0113] A consensus sequence for the mutant ilvC was generated from the multiple sequence alignment and is provided as SEQ ID NO: 29 which represents all experimentally verified mutations of the KARI enzyme based on the amino acid sequence of the KARI enzyme isolated from *Pseudomonas fluorescens*, (SEQ ID NO:17)

[0114] Additionally the present invention describes mutation positions identified using a profile Hidden Markov Model (HMM) built based on sequences of 25 functionally verified Class I and Class II KARI enzymes. Profile HMM identified mutation positions 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, and 170 (the numbering is based on the sequences of *Pseudomonas fluorescens* PF5 KARI). Thus, it will be appreciated by the skilled person that mutations at these positions, as well as those discussed above that have been experimentally verified will also give rise to KARI enzymes having the ability to bind NADH.

The Host Strains for Kari Engineering

[0115] Two host strains, *E. coli* TOP10 from Invitrogen and *E. Coli* Bw25113 ( $\Delta$ ilvC, an ilvC gene-knockout), were used

for making constructs over-expressing the KARI enzyme in this disclosure. In the Bw25113 strain, the entire *ilvC* gene of the *E. Coli* chromosome was replaced by a Kanamycin cassette using the Lambda red homologous recombination technology described by Kirill et al., (Kirill A. Datsenko and Barry L. Wanner, *Proc. Natl. Acad. Sci. USA*, 97, 6640-6645, 2000). Homology Modeling of Pf5 Kari with Bound Substrates

**[0116]** The structure of PF5-KARI with bound NADPH, acetolactate and magnesium ions was built based on the crystal structure of *P. aeruginosa* PAO1-KARI (PDB ID 1 NP3, Ahn H. J. et al. *J. Mol. Biol.*, 328, 505-515, 2003) which has 92% amino acid sequence homology to PF5 KARI. PAO1-KARI structure is a homo-dodecamer and each dodecamer consists of six homo-dimers with extensive dimer interface. The active site of KARI is located in this dimer interface. The biological assembly is formed by six homo-dimers positioned on the edges of a tetrahedron resulting in a highly symmetrical dodecamer of 23 point group symmetry. For simplicity, only the dimeric unit (monomer A and monomer B) was built for the homology model of PF5-KARI in this study because the active site is in the homo-dimer interface.

**[0117]** The model of PF5-KARI dimer was built based on the coordinates of monomer A and monomer B of PAO1-KARI and sequence of PF5-KARI using DeepView/Swiss PDB viewer (Guex, N. and Peitsch, M. C. *Electrophoresis* 18: 2714-2723, 1997). This model was then imported to program O (Jones, T. A. et al, *Acta Crystallogr. A* 47, 110-119, 1991) on a Silicon Graphics system for further modification.

**[0118]** The structure of PAO1-KARI has no NADPH, substrate or inhibitor or magnesium in the active site. Therefore, the spinach KARI structure (PDB ID 1yve, Biou V. et al. *The EMBO Journal*, 16: 3405-3415, 1997.), which has magnesium ions, NADPH and inhibitor (N-Hydroxy-N-isopropylloxamate) in the acetolactate binding site, was used to model these molecules in the active site. The plant KARI has very little sequence homology to either PF5- or PAO1 KARI (<20% amino acid identity), however the structures in the active site region of these two KARI enzymes are very similar. To overlay the active site of these two KARI structures, commands LSQ\_ext, LSQ\_improve, LSQ\_mol in the program O were used to line up the active site of monomer A of spinach KARI to the monomer A of PF5 KARI model. The coordinates of NADPH, two magnesium ions and the inhibitor bound in the active site of spinach KARI were extracted and incorporated to molecule A of PF5 KARI. A set of the coordinates of these molecules were generated for monomer B of PF5 KARI by applying the transformation operator from monomer A to monomer B calculated by the program.

**[0119]** Because there is no NADPH in the active site of PAO1 KARI crystal structure, the structures of the phosphate binding loop region in the NADPH binding site (residues 44-45 in PAO1 KARI, 157-170 in spinach KARI) are very different between the two. To model the NADPH bound form, the model of the PF5-KARI phosphate binding loop (44-55) was replaced by that of 1yve (157-170). Any discrepancy of side chains between these two was converted to those in the PF5-KARI sequence using the mutate\_replace command in program O, and the conformations of the replaced side-chains were manually adjusted. The entire NADPH/Mg/inhibitor bound dimeric PF5-KARI model went through one round of energy minimization using program CNX (ACCELRYLS San Diego Calif., Burnger, A. T. and Warren, G. L., *Acta Crystallogr.*, D 54, 905-921, 1998) after which the inhibitor was replaced by the substrate, acetolactate (AL), in the model.

The conformation of AL was manually adjusted to favor hydride transfer of C4 of the nicotinamide of NADPH and the substrate. No further energy minimization was performed on this model (Coordinates of the model created for this study are attached in a separate word file.). The residues in the phosphate binding loop and their interactions with NADPH are illustrated in FIG. 3.

Application of a Profile Hidden Markov Model for Identification of Residue Positions Involved in Cofactor Switching in Kari Enzymes

**[0120]** Applicants have developed a method for identifying KARI enzymes and the residue positions that are involved in cofactor switching from NADPH to NADH. To structurally characterize KARI enzymes, a Profile Hidden Markov Model (HMM) was prepared as described in Example 5 using amino acid sequences of 25 KARI proteins with experimentally verified function as outlined in Table 6. These KARIs were from [*Pseudomonas fluorescens* Pf-5 (SEQ ID NO: 17), *Sulfolobus solfataricus* P2 (SEQ ID NO: 13), *Pyrobaculum aerophilum* str. IM2 (SEQ ID NO: 14), *Natronomonas pharaonis* DSM 2160 (SEQ ID NO: 30), *Bacillus subtilis* subsp. *subtilis* str. 168 (SEQ ID NO: 31), *Corynebacterium glutamicum* ATCC 13032 (SEQ ID NO: 32), *Phaeosporium molischianum* (SEQ ID NO: 33), *Ralstonia solanacearum* GMI1000 (SEQ ID NO: 15), *Zymomonas mobilis* subsp. *mobilis* ZM4 (SEQ ID NO: 34), *Alkalilimnicola ehrlichei* MLHE-1 (SEQ ID NO: 35), *Campylobacter lari* RM2100 (SEQ ID NO: 36), *Marinobacter aquaeolei* VT8 (SEQ ID NO: 37), *Psychrobacter arcticus* 273-4 (SEQ ID NO: 38), *Hahella chejuensis* KCTC 2396 (SEQ ID NO: 39), *Thiobacillus denitrificans* ATCC 25259 (SEQ ID NO: 40), *Azotobacter vinelandii* AVOP (SEQ ID NO: 41), *Pseudomonas syringae* pv. *syringae* B728a (SEQ ID NO: 42), *Pseudomonas syringae* pv. *tomato* str. DC3000 (SEQ ID NO: 43), *Pseudomonas putida* KT2440 (Protein SEQ ID NO: 44), *Pseudomonas entomophila* L48 (SEQ ID NO: 45), *Pseudomonas mendocina* ymp (SEQ ID NO: 46), *Pseudomonas aeruginosa* PAO1 (SEQ ID NO: 16), *Bacillus cereus* ATCC 10987 (SEQ ID NO: 47), *Bacillus cereus* ATCC 10987 (SEQ ID NO: 48), and *Spinacia oleracea* (SEQ ID NO: 18)].

**[0121]** In addition using methods disclosed in this application, sequences of Class II KARI enzymes such as *E. coli* (SEQ ID NO: 63-GenBank Accession Number P05793), marine gamma Proteobacterium HTCC2207 (SEQ ID NO: 64-GenBank Accession Number ZP\_01224863.1), *Desulfuromonas acetoxidans* (SEQ ID NO: 65-GenBank Accession Number ZP\_01313517.1) and *Pisum sativum* (pea) (SEQ ID NO: 66-GenBank Accession Number 082043) could be mentioned.

**[0122]** This Profile HMM for KARIs can be used to identify any KARI related proteins. Any protein that matches the Profile HMM with an E value of  $<10^{-3}$  using hmmsearch program in the HMMER package is expected to be a functional KARI, which can be either a Class I and Class II KARI. Sequences matching the Profile HMM given herein are then analyzed for the location of the 12 positions in *Pseudomonas fluorescens* Pf-5 that switches the cofactor from NADPH to NADH. The eleven nodes, as defined in the section of Profile HMM building, in the profile HMM representing the columns in the alignment which correspond to the eleven co-factor switching positions in *Pseudomonas fluorescens* Pf-5 KARI are identified as node 24, 33, 47, 50, 52, 53, 61, 80, 115, 156 and 170. The lines corresponding to these nodes in the model

file are identified in Table 9. One skilled in the art will readily be able to identify these 12 positions in the amino acid sequence of a KARI protein from the alignment of the sequence to the profile HMM using hmmsearch program in HMMER package.

**[0123]** The KARI enzymes identified by this method, include both Class I and Class II KARI enzymes from either microbial or plant natural sources. Any KARI identified by this method may be used for heterologous expression in microbial cells.

**[0124]** For example each of the KARI encoding nucleic acid fragments described herein may be used to isolate genes encoding homologous proteins. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to: 1) methods of nucleic acid hybridization; 2) methods of DNA and RNA amplification, as exemplified by various uses of nucleic acid amplification technologies [e.g., polymerase chain reaction (PCR), Mullis et al., U.S. Pat. No. 4,683,202; ligase chain reaction (LCR), Tabor, S. et al., *Proc. Acad. Sci. USA* 82:1074 (1985); or strand displacement amplification (SDA), Walker, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 89:392 (1992)]; and 3.) methods of library construction and screening by complementation.

**[0125]** Although the sequence homology between Class I and Class II KARI enzymes is low, the three dimensional structure of both Classes of the enzymes, particularly around the active site and nucleotide binding domains is highly conserved (Tygai, R., et al., *Protein Science*, 34: 399-408, 2001). The key amino acid residues that make up the substrate binding pocket are highly conserved between these two Classes even though they may not align well in a simple sequence comparison. It can therefore be concluded that the residues affecting cofactor specificity identified in Class I KARI (e.g., positions 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, and 170 of PF5 KARI) can be extended to Class II KARI enzymes.

#### Isobutanol Biosynthetic Pathways

**[0126]** Carbohydrate utilizing microorganisms employ the Embden-Meyerhof-Parnas (EMP) pathway, the Entner and Doudoroff pathway and the pentose phosphate cycle as the central, metabolic routes to provide energy and cellular precursors for growth and maintenance. These pathways have in common the intermediate glyceraldehyde-3-phosphate and, ultimately, pyruvate is formed directly or in combination with the EMP pathway. Subsequently, pyruvate is transformed to acetyl-cofactor A (acetyl-CoA) via a variety of means. Acetyl-CoA serves as a key intermediate, for example, in generating fatty acids, amino acids and secondary metabolites. The combined reactions of sugar conversion to pyruvate produce energy (e.g. adenosine-5'-triphosphate, ATP) and reducing equivalents (e.g. reduced nicotinamide adenine dinucleotide, NADH, and reduced nicotinamide adenine dinucleotide phosphate, NADPH). NADH and NADPH must be recycled to their oxidized forms (NAD<sup>+</sup> and NADP<sup>+</sup>, respectively). In the presence of inorganic electron acceptors (e.g. O<sub>2</sub>, NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>), the reducing equivalents may be used to augment the energy pool; alternatively, a reduced carbon byproduct may be formed.

**[0127]** There are four potential pathways for production of isobutanol from carbohydrate sources with recombinant microorganisms as shown in FIG. 1. All potential pathways for conversion of carbohydrates to isobutanol have been

described in the commonly owned U.S. patent application Ser. No. 11/586,315, which is incorporated herein by reference.

**[0128]** The preferred pathway for conversion of pyruvate to isobutanol consists of enzymatic steps "a", "b", "c", "d", and "e" (FIG. 1) and includes the following substrate to product conversions:

**[0129]** a) pyruvate to acetolactate, as catalyzed for example by acetolactate synthase,

**[0130]** b) (S)-acetolactate to 2,3-dihydroxyisovalerate, as catalyzed for example by acetohydroxy acid isomeroeductase,

**[0131]** c) 2,3-dihydroxyisovalerate to  $\alpha$ -ketoisovalerate, as catalyzed for example by acetohydroxy acid dehydratase,

**[0132]** d)  $\alpha$ -ketoisovalerate to isobutyraldehyde, as catalyzed for example by a branched-chain keto acid decarboxylase, and

**[0133]** e) isobutyraldehyde to isobutanol, as catalyzed for example by, a branched-chain alcohol dehydrogenase.

**[0134]** This pathway combines enzymes involved in well-characterized pathways for valine biosynthesis (pyruvate to  $\alpha$ -ketoisovalerate) and valine catabolism  $\alpha$ -ketoisovalerate to isobutanol). Since many valine biosynthetic enzymes also catalyze analogous reactions in the isoleucine biosynthetic pathway, substrate specificity is a major consideration in selecting the gene sources. For this reason, the primary genes of interest for the acetolactate synthase enzyme are those from *Bacillus* (alsS) and *Klebsiella* (budB). These particular acetolactate synthases are known to participate in butanediol fermentation in these organisms and show increased affinity for pyruvate over ketobutyrate (Gollup et al., *J. Bacteriol.* 172, 3444-3449, 1990); and (Holtzclaw et al., *J. Bacteriol.* 121, 917-922, 1975). The second and third pathway steps are catalyzed by acetohydroxy acid reductoisomerase and dehydratase, respectively. These enzymes have been characterized from a number of sources, such as for example, *E. coli* (Chunduru et al., *Biochemistry* 28, 486-493, 1989); and (Flint et al., *J. Biol. Chem.* 268, 14732-14742, 1993). The final two steps of the preferred isobutanol pathway are known to occur in yeast, which can use valine as a nitrogen source and, in the process, secrete isobutanol.  $\alpha$ -Ketoisovalerate can be converted to isobutyraldehyde by a number of keto acid decarboxylase enzymes, such as for example pyruvate decarboxylase. To prevent misdirection of pyruvate away from isobutanol production, a decarboxylase with decreased affinity for pyruvate is desired. So far, there are two such enzymes known in the art (Smit et al., *Appl. Environ. Microbiol.*, 71, 303-311, 2005); and (de la Plaza et al., *FEMS Microbiol. Lett.*, 238, 367-374, 2004). Both enzymes are from strains of *Lactococcus lactis* and have a 50-200-fold preference for ketoisovalerate over pyruvate. Finally, a number of aldehyde reductases have been identified in yeast, many with overlapping substrate specificity. Those known to prefer branched-chain substrates over acetaldehyde include, but are not limited to, alcohol dehydrogenase VI (ADH6) and Ypr1p (Larroy et al., *Biochem. J.* 361, 163-172, 2002); and (Ford et al., *Yeast* 19, 1087-1096, 2002), both of which use NADPH as electron donor. An NADPH-dependent reductase, YqhD, active with branched-chain substrates has also been recently identified in *E. coli* (Sulzenbacher et al., *J. Mol. Biol.* 342, 489-502, 2004).

**[0135]** Two of the other potential pathways for isobutanol production also contain the initial three steps of "a", "b" and

“c” (FIG. 1). One pathway consists of enzymatic steps “a”, “b”, “c”, “f”, “g”, “e” (FIG. 1). Step “f” containing a “branched-chain keto acid dehydrogenase” with an EC number 1.2.4.4. Step “g” containing an “acylating aldehyde dehydrogenase” with a EC numbers 1.2.1.10 and 1.2.1.57 in addition to step “e” containing the “branched chain alcohol dehydrogenase”. The other potential pathway consists of steps “a”, “b”, “c”, “h”, “i”, “j”, (FIG. 1). The term “transaminase” (step “h”) EC numbers 2.6.1.42 and 2.6.1.66. Step “h” consists of either a “valine dehydrogenase” with EC numbers 1.4.1.8 and 1.4.1.9 or step “i”, a “valine decarboxylase” with an EC number 4.1.1.14. Finally step “j” will use an “omega transaminase” with an EC number 2.6.1.18 to generate isobutyraldehyde which will be reduced by step “e” to produce isobutanol. All potential pathways for conversion of pyruvate to isobutanol are depicted in FIG. 1.

[0136] Additionally, a number of organisms are known to produce butyrate and/or butanol via a butyryl-CoA intermediate (Dürre, et al., *FEMS Microbiol. Rev.* 17, 251-262, 1995); and (Abbad-Andaloussi et al., *Microbiology* 142, 1149-1158, 1996). Therefore isobutanol production in these organisms will take place using steps “k”, “g” and “e” shown in FIG. 1. Step “k” will use an “isobutyryl-CoA mutase” with an EC number 5.4.99.13. The next step will involve using the “acylating aldehyde dehydrogenase” with the EC numbers 1.2.1.10 and 1.2.1.57 to produce isobutyraldehyde followed by enzymatic step “e” to produce isobutanol. All these pathways are fully described in the commonly owned patent application CL3243 Herein incorporated by reference.

[0137] Thus, in providing multiple recombinant pathways from pyruvate to isobutanol, there exist a number of choices to fulfill the individual conversion steps, and the person of skill in the art will be able to utilize publicly available sequences to construct the relevant pathways.

#### Microbial Hosts for Isobutanol Production

[0138] Microbial hosts for isobutanol production may be selected from bacteria, cyanobacteria, filamentous fungi and yeasts. The microbial host used for isobutanol production should be tolerant to isobutanol so that the yield is not limited by butanol toxicity. Microbes that are metabolically active at high titer levels of isobutanol are not well known in the art. Although butanol-tolerant mutants have been isolated from solventogenic Clostridia, little information is available concerning the butanol tolerance of other potentially useful bacterial strains. Most of the studies on the comparison of alcohol tolerance in bacteria suggest that butanol is more toxic than ethanol (de Cavalho, et al., *Microsc. Res. Tech.* 64, 215-22, 2004) and (Kabelitz, et al., *FEMS Microbiol. Lett.* 220, 223-227, 2003, Tomas, et al. *J. Bacteriol.* 186, 2006-2018, 2004) report that the yield of 1-butanol during fermentation in *Clostridium acetobutylicum* may be limited by 1-butanol toxicity. The primary effect of 1-butanol on *Clostridium acetobutylicum* is disruption of membrane functions (Hermann et al., *Appl. Environ. Microbiol.* 50, 1238-1243, 1985).

[0139] The microbial hosts selected for the production of isobutanol should be tolerant to isobutanol and should be able to convert carbohydrates to isobutanol. The criteria for selection of suitable microbial hosts include the following: intrinsic tolerance to isobutanol, high rate of glucose utilization, availability of genetic tools for gene manipulation, and the ability to generate stable chromosomal alterations.

[0140] Suitable host strains with a tolerance for isobutanol may be identified by screening based on the intrinsic toler-

ance of the strain. The intrinsic tolerance of microbes to isobutanol may be measured by determining the concentration of isobutanol that is responsible for 50% inhibition of the growth rate ( $IC_{50}$ ) when grown in a minimal medium. The  $IC_{50}$  values may be determined using methods known in the art. For example, the microbes of interest may be grown in the presence of various amounts of isobutanol and the growth rate monitored by measuring the optical density at 600 nanometers. The doubling time may be calculated from the logarithmic part of the growth curve and used as a measure of the growth rate. The concentration of isobutanol that produces 50% inhibition of growth may be determined from a graph of the percent inhibition of growth versus the isobutanol concentration. Preferably, the host strain should have an  $IC_{50}$  for isobutanol of greater than about 0.5%.

[0141] The microbial host for isobutanol production should also utilize glucose at a high rate. Most microbes are capable of utilizing carbohydrates. However, certain environmental microbes cannot utilize carbohydrates to high efficiency, and therefore would not be suitable hosts.

[0142] The ability to genetically modify the host is essential for the production of any recombinant microorganism. The mode of gene transfer technology may be by electroporation, conjugation, transduction or natural transformation. A broad range of host conjugative plasmids and drug resistance markers are available. The cloning vectors are tailored to the host organisms based on the nature of antibiotic resistance markers that can function in that host.

[0143] The microbial host also has to be manipulated in order to inactivate competing pathways for carbon flow by deleting various genes. This requires the availability of either transposons to direct inactivation or chromosomal integration vectors. Additionally, the production host should be amenable to chemical mutagenesis so that mutations to improve intrinsic isobutanol tolerance may be obtained.

[0144] Based on the criteria described above, suitable microbial hosts for the production of isobutanol include, but are not limited to, members of the genera *Clostridium*, *Zymomonas*, *Escherichia*, *Salmonella*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Vibrio*, *Lactobacillus*, *Enterococcus*, *Alcaligenes*, *Klebsiella*, *Paenibacillus*, *Arthrobacter*, *Corynebacterium*, *Brevibacterium*, *Pichia*, *Candida*, *Hansenula* and *Saccharomyces*. Preferred hosts include: *Escherichia coli*, *Alcaligenes eutrophus*, *Bacillus licheniformis*, *Paenibacillus macerans*, *Rhodococcus erythropolis*, *Pseudomonas putida*, *Lactobacillus plantarum*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus faecalis*, *Bacillus subtilis* and *Saccharomyces cerevisiae*.

#### Construction of Production Host

[0145] Recombinant organisms containing the necessary genes that will encode the enzymatic pathway for the conversion of a fermentable carbon substrate to isobutanol may be constructed using techniques well known in the art. In the present invention, genes encoding the enzymes of one of the isobutanol biosynthetic pathways of the invention, for example, acetolactate synthase, acetohydroxy acid isomeroeductase, acetohydroxy acid dehydratase, branched-chain  $\alpha$ -keto acid decarboxylase, and branched-chain alcohol dehydrogenase, may be isolated from various sources, as described above.

[0146] Methods of obtaining desired genes from a bacterial genome are common and well known in the art of molecular biology. For example, if the sequence of the gene is known,



suitable genomic libraries may be created by restriction endonuclease digestion and may be screened with probes complementary to the desired gene sequence. Once the sequence is isolated, the DNA may be amplified using standard primer-directed amplification methods such as polymerase chain reaction (U.S. Pat. No. 4,683,202) to obtain amounts of DNA suitable for transformation using appropriate vectors. Tools for codon optimization for expression in a heterologous host are readily available. Some tools for codon optimization are available based on the GC content of the host organism.

**[0147]** Once the relevant pathway genes are identified and isolated they may be transformed into suitable expression hosts by means well known in the art. Vectors or cassettes useful for the transformation of a variety of host cells are common and commercially available from companies such as EPICENTRE® (Madison, Wis.), Invitrogen Corp. (Carlsbad, Calif.), Stratagene (La Jolla, Calif.), and New England Biolabs, Inc. (Beverly, Mass.). Typically the vector or cassette contains sequences directing transcription and translation of the relevant gene, a selectable marker, and sequences allowing autonomous replication or chromosomal integration. Suitable vectors comprise a region 5' of the gene which harbors transcriptional initiation controls and a region 3' of the DNA fragment which controls transcriptional termination. 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.

**[0148]** Initiation control regions or promoters, which are useful to drive expression of the relevant pathway coding regions in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genetic elements is suitable for the present invention including, but not limited to, CYC1, HIS3, GAL1, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI (useful for expression in *Saccharomyces*); AOX1 (useful for expression in *Pichia*); and lac, ara, tet, trp, IP<sub>L</sub>, IP<sub>R</sub>, T7, tac, and trc (useful for expression in *Escherichia coli*, *Alcaligenes*, and *Pseudomonas*) as well as the amy, apr, npr promoters and various phage promoters useful for expression in *Bacillus subtilis*, *Bacillus licheniformis*, and *Paenibacillus macerans*.

**[0149]** Termination control regions may also be derived from various genes native to the preferred hosts. Optionally, a termination site may be unnecessary, however, it is most preferred if included.

**[0150]** Certain vectors are capable of replicating in a broad range of host bacteria and can be transferred by conjugation. The complete and annotated sequence of pRK404 and three related vectors-pRK437, pRK442, and pRK442(H) are available. These derivatives have proven to be valuable tools for genetic manipulation in Gram-negative bacteria (Scott et al., Plasmid 50, 74-79, 2003). Several plasmid derivatives of broad-host-range Inc P4 plasmid RSF1010 are also available with promoters that can function in a range of Gram-negative bacteria. Plasmid pAYC36 and pAYC37, have active promoters along with multiple cloning sites to allow for the heterologous gene expression in Gram-negative bacteria.

**[0151]** Chromosomal gene replacement tools are also widely available. For example, a thermosensitive variant of the broad-host-range replicon pWV101 has been modified to construct a plasmid pVE6002 which can be used to effect gene replacement in a range of Gram-positive bacteria

(Maguin et al., *J. Bacteriol.* 174, 5633-5638, 1992). Additionally, in vitro transposomes are available to create random mutations in a variety of genomes from commercial sources such as EPICENTRE®.

**[0152]** The expression of an isobutanol biosynthetic pathway in various preferred microbial hosts is described in more detail below.

Expression of an Isobutanol Biosynthetic Pathway in *E. Coli*

**[0153]** Vectors or cassettes useful for the transformation of *E. coli* are common and commercially available from the companies listed above. For example, the genes of an isobutanol biosynthetic pathway may be isolated from various sources, cloned into a modified pUC19 vector and transformed into *E. coli* NM522.

Expression of an Isobutanol Biosynthetic Pathway in *Rhodococcus Erythropolis*

**[0154]** A series of *E. coli*-*Rhodococcus* shuttle vectors are available for expression in *R. erythropolis*, including, but not limited to, pRhBR17 and pDA71 (Kostichka et al., *Appl. Microbiol. Biotechnol.* 62, 61-68, 2003). Additionally, a series of promoters are available for heterologous gene expression in *R. erythropolis* (Nakashima et al., *Appl. Environ. Microbiol.* 70, 5557-5568, 2004 and Tao et al., *Appl. Microbiol. Biotechnol.* 68, 346-354, 2005). Targeted gene disruption of chromosomal genes in *R. erythropolis* may be created using the method described by Tao et al., supra, and Brans et al. (*Appl. Environ. Microbiol.* 66, 2029-2036, 2000).

**[0155]** The heterologous genes required for the production of isobutanol, as described above, may be cloned initially in pDA71 or pRhBR71 and transformed into *E. coli*. The vectors may then be transformed into *R. erythropolis* by electroporation, as described by Kostichka et al., supra. The recombinants may be grown in synthetic medium containing glucose and the production of isobutanol can be followed using methods known in the art.

Expression of an Isobutanol Biosynthetic Pathway in *B. Subtilis*

**[0156]** Methods for gene expression and creation of mutations in *B. subtilis* are also well known in the art. For example, the genes of an isobutanol biosynthetic pathway may be isolated from various sources, cloned into a modified pUC19 vector and transformed into *Bacillus subtilis* BE1010. Additionally, the five genes of an isobutanol biosynthetic pathway can be split into two operons for expression. The three genes of the pathway (bubB, ilvD, and kivD) can be integrated into the chromosome of *Bacillus subtilis* BE1010 (Payne, et al., *J. Bacteriol.* 173, 2278-2282, 1991). The remaining two genes (ilvC and bdhB) can be cloned into an expression vector and transformed into the *Bacillus* strain carrying the integrated isobutanol genes

Expression of an Isobutanol Biosynthetic Pathway in *B. Licheniformis*

**[0157]** Most of the plasmids and shuttle vectors that replicate in *B. subtilis* may be used to transform *B. licheniformis* by either protoplast transformation or electroporation. The genes required for the production of isobutanol may be cloned in plasmids pBE20 or pBE60 derivatives (Nagarajan et al., *Gene* 114,121-126, 1992). Methods to transform *B. licheniformis* are known in the art (Fleming et al. *Appl. Environ.*



Microbiol., 61, 3775-3780, 1995). The plasmids constructed for expression in *B. subtilis* may be transformed into *B. licheniformis* to produce a recombinant microbial host that produces isobutanol.

Expression of an Isobutanol Biosynthetic Pathway in *Paenibacillus Maceans*

**[0158]** Plasmids may be constructed as described above for expression in *B. subtilis* and used to transform *Paenibacillus maceans* by protoplast transformation to produce a recombinant microbial host that produces isobutanol.

Expression of the Isobutanol Biosynthetic Pathway in *Alcaligenes (Ralstonia) Eutrophus*

**[0159]** Methods for gene expression and creation of mutations in *Alcaligenes eutrophus* are known in the art (Taghavi et al., *Appl. Environ. Microbiol.*, 60, 3585-3591, 1994). The genes for an isobutanol biosynthetic pathway may be cloned in any of the broad host range vectors described above, and electroporated to generate recombinants that produce isobutanol. The poly(hydroxybutyrate) pathway in *Alcaligenes* has been described in detail, a variety of genetic techniques to modify the *Alcaligenes eutrophus* genome is known, and those tools can be applied for engineering an isobutanol biosynthetic pathway.

Expression of an isobutanol biosynthetic pathway in *Pseudomonas putida*

**[0160]** Methods for gene expression in *Pseudomonas putida* are known in the art (see for example Ben-Bassat et al., U.S. Pat. No. 6,586,229, which is incorporated herein by reference). The butanol pathway genes may be inserted into pPCU18 and this ligated DNA may be electroporated into electrocompetent *Pseudomonas putida* DOT-T1C5aAR1 cells to generate recombinants that produce isobutanol.

Expression of an Isobutanol Biosynthetic Pathway in *Saccharomyces Cerevisiae*

**[0161]** Methods for gene expression in *Saccharomyces cerevisiae* are known in the art (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, Calif.). Expression of genes in yeast typically requires a promoter, followed by the gene of interest, and a transcriptional terminator. A number of yeast promoters can be used in constructing expression cassettes for genes encoding an isobutanol biosynthetic pathway, including, but not limited to constitutive promoters FBA, GPD, ADH1, and GPM, and the inducible promoters GAL1, GAL10, and CUP1. Suitable transcriptional terminators include, but are not limited to FBA<sub>t</sub>, GPD<sub>t</sub>, GPM<sub>t</sub>, ERG10<sub>t</sub>, GAL1<sub>t</sub>, CYC1, and ADH1. For example, suitable promoters, transcriptional terminators, and the genes of an isobutanol biosynthetic pathway may be cloned into *E. coli*-yeast shuttle vectors.

Expression of an Isobutanol Biosynthetic Pathway in *Lactobacillus Plantarum*

**[0162]** The *Lactobacillus* genus belongs to the *Lactobacillus* family and many plasmids and vectors used in the transformation of *Bacillus subtilis* and *Streptococcus* may be used for *lactobacillus*. Non-limiting examples of suitable vectors include pAMβ1 and derivatives thereof (Renault et al., *Gene* 183,175-182, 1996); and (O'Sullivan et al., *Gene* 137, 227-

231, 1993); pMBB1 and pHW800, a derivative of pMBB1 (Wyckoff et al., *Appl. Environ. Microbiol.* 62, 1481-1486, 1996); pMG1, a conjugative plasmid (Tanimoto et al., *J. Bacteriol.* 184, 5800-5804, 2002); pNZ9520 (Kleerebezem et al., *Appl. Environ. Microbiol.* 63, 4581-4584, 1997); pAM401 (Fujimoto et al., *Appl. Environ. Microbiol.* 67, 1262-1267, 2001); and pAT392 (Arthur et al., *Antimicrob. Agents Chemother.* 38, 1899-1903, 1994). Several plasmids from *Lactobacillus plantarum* have also been reported (van Kranenburg R, et al. *Appl. Environ. Microbiol.* 71, 1223-1230, 2005).

Expression of an Isobutanol Biosynthetic Pathway in Various *Enterococcus* species (*E. faecium*, *E. gallinarium*, and *E. faecalis*)

**[0163]** The *Enterococcus* genus belongs to the *Lactobacillus* family and many plasmids and vectors used in the transformation of *Lactobacilli*, *Bacilli* and *Streptococci* species may be used for *Enterococcus* species. Non-limiting examples of suitable vectors include pAMβ1 and derivatives thereof (Renault et al., *Gene* 183,175-182, 1996); and (O'Sullivan et al., *Gene* 137, 227-231, 1993); pMBB1 and pHW800, a derivative of pMBB1 (Wyckoff et al. *Appl. Environ. Microbiol.* 62, 1481-1486, 1996); pMG1, a conjugative plasmid (Tanimoto et al., *J. Bacteriol.* 184, 5800-5804, 2002); pNZ9520 (Kleerebezem et al., *Appl. Environ. Microbiol.* 63, 4581-4584, 1997); pAM401 (Fujimoto et al., *Appl. Environ. Microbiol.* 67, 1262-1267, 2001); and pAT392 (Arthur et al., *Antimicrob. Agents Chemother.* 38, 1899-1903, 1994). Expression vectors for *E. faecalis* using the nisA gene from *Lactococcus* may also be used (Eichenbaum et al., *Appl. Environ. Microbiol.* 64, 2763-2769, 1998). Additionally, vectors for gene replacement in the *E. faecium* chromosome may be used (Nallaapareddy et al., *Appl. Environ. Microbiol.* 72, 334-345, 2006).

Fermentation Media

**[0164]** Fermentation media in the present invention must contain suitable carbon substrates. Suitable substrates may include but are not limited to monosaccharides such as glucose and fructose, oligosaccharides such as lactose or sucrose, polysaccharides such as starch or cellulose or mixtures thereof and unpurified mixtures from renewable feedstocks such as cheese whey permeate, cornsteep liquor, sugar beet molasses, and barley malt. Additionally the carbon substrate may also be one-carbon substrates such as carbon dioxide, or methanol for which metabolic conversion into key biochemical intermediates has been demonstrated. In addition to one and two carbon substrates methylotrophic organisms are also known to utilize a number of other carbon containing compounds such as methylamine, glucosamine and a variety of amino acids for metabolic activity. For example, methylotrophic yeast 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. (eds): 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.

**[0165]** Although it is contemplated that all of the above mentioned carbon substrates and mixtures thereof are suit-

able in the present invention, preferred carbon substrates are glucose, fructose, and sucrose.

[0166] In addition to an appropriate carbon source, fermentation media must contain suitable minerals, salts, cofactors, buffers and other components, known to those skilled in the art, suitable for the growth of the cultures and promotion of the enzymatic pathway necessary for isobutanol production.

#### Culture Conditions

[0167] Typically cells are grown at a temperature in the range of about 25° C. to about 40° C. in an appropriate medium. Suitable growth media in the present invention are common commercially prepared media such as Luria Bertani (LB) broth, Sabouraud Dextrose (SD) broth or Yeast Medium (YM) broth. Other defined or synthetic growth media may also be used, and the appropriate medium for growth of the particular microorganism will be known by one skilled in the art of microbiology or fermentation science. The use of agents known to modulate catabolite repression directly or indirectly, e.g., cyclic adenosine 2',3'-monophosphate (cAMP), may also be incorporated into the fermentation medium.

[0168] Suitable pH ranges for the fermentation are between pH 5.0 to pH 9.0, where pH 6.0 to pH 8.0 is preferred for the initial condition.

[0169] Fermentations may be performed under aerobic or anaerobic conditions, where anaerobic or microaerobic conditions are preferred.

#### Industrial Batch and Continuous Fermentations

[0170] The present process employs a batch method of fermentation. A classical batch fermentation is a closed system where the composition of the medium is set at the beginning of the fermentation and not subject to artificial alterations during the fermentation. Thus, at the beginning of the fermentation the medium is inoculated with the desired organism or organisms, and fermentation is permitted to occur without adding anything to the system. Typically, however, a "batch" fermentation is batch with respect to the addition of carbon source and attempts are often made at controlling factors such as pH and oxygen concentration. In batch systems the metabolite and biomass compositions of the system change constantly up to the time the fermentation is stopped. Within batch cultures cells moderate through a static lag phase to a high growth log phase and finally to a stationary phase where growth rate is diminished or halted. If untreated, cells in the stationary phase will eventually die. Cells in log phase generally are responsible for the bulk of production of end product or intermediate.

[0171] 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. Measurement of the actual substrate concentration in Fed-Batch systems is difficult and is therefore estimated on the basis of the changes of measurable factors such as pH, dissolved oxygen and the partial pressure of waste gases such as CO<sub>2</sub>. Batch and Fed-Batch fermentations are common and well known in the art and examples may be found in Thomas D. Brock in *Biotechnol-*

*ogy: A Textbook of Industrial Microbiology*, Second Edition (1989) Sinauer Associates, Inc., Sunderland, Mass., or Deshpande, Mukund (*Appl. Biochem. Biotechnol.*, 36, 227, 1992), herein incorporated by reference.

[0172] Although the present invention is performed in batch mode it is contemplated that the method would be adaptable to continuous fermentation methods. Continuous fermentation is an open system where a defined fermentation medium is added continuously to a bioreactor and an equal amount of conditioned media is removed simultaneously for processing. Continuous fermentation generally maintains the cultures at a constant high density where cells are primarily in log phase growth.

[0173] Continuous fermentation allows for modulation of one factor or any number of factors that affect cell growth or end product concentration. For example, one method will maintain a limiting nutrient such as the carbon source or nitrogen level at a fixed rate and allow all other parameters to moderate. In other systems a number of factors affecting growth can be altered continuously while the cell concentration, measured by media turbidity, is kept constant. Continuous systems strive to maintain steady state growth conditions and thus the cell loss due to the medium being drawn off must be balanced against the cell growth rate in the fermentation. Methods of modulating nutrients and growth factors for continuous fermentation processes as well as 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.

[0174] It is contemplated that the present invention may be practiced using either 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. Methods for Isobutanol Isolation from the Fermentation Medium

[0175] The biologically produced isobutanol may be isolated from the fermentation medium using methods known in the art for Acetone-butanol-ethanol (ABE) fermentations (see for example, Durre, *Appl. Microbiol. Biotechnol.* 49, 639-648, 1998), and (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 and 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.

#### EXAMPLES

[0176] The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various uses and conditions.

#### General Methods:

[0177] 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. Bannan, 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. 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*, Phillip 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, D.C., 1994, or by Thomas D. Brock in *Biotechnology: A Textbook of Industrial Microbiology*, Second Edition, Sinauer Associates, Inc., Sunderland, Mass., 1989. All reagents, restriction enzymes and materials used for the growth and maintenance of bacterial cells were obtained from Aldrich Chemicals (Milwaukee, Wis.), BD Diagnostic Systems (Sparks, Md.), Life Technologies (Rockville, Md.), or Sigma Chemical Company (St. Louis, Mo.), unless otherwise specified.

**[0178]** The meaning of abbreviations used is as follows: "Å" means Angstrom, "min" means minute(s), "h" means hour(s), "µl" means microliter(s), "ng/µl" means nano gram per microliter, "pmol/µl" means pico mole per microliter, "ml" means milliliter(s), "L" means liter(s), "g/L" mean gram per liter, "ng" means nano gram, "sec" means second(s), "ml/min" means milliliter per minute(s), "w/v" means weight per volume, "v/v" means volume per volume, "nm" means nanometer(s), "mm" means millimeter(s), "cm" means centimeter(s) "mM" means millimolar, "M" means molar, "µmol" means millimole(s), "µmole" means micromole(s), "g" means gram(s), "µg" means microgram(s), "mg" means milligram(s), "g" means the gravitation constant, "rpm" means revolutions per minute, "HPLC" means high performance liquid chromatography, "MS" means mass spectrometry, "HPLC/MS" means high performance liquid chromatography/mass spectrometry, "EDTA" means ethylenediamine-tetraacetic acid, "dNTP" means deoxynucleotide triphosphate.

**[0179]** The oligonucleotide primers used in the following Examples have been described herein (see Table 1)

#### High Throughput Screening Assay of Gene Libraries

**[0180]** High throughput screening of the gene libraries of mutant KARI enzymes was performed as described herein: 10× freezing medium containing 554.4 g/L glycerol, 68 mM of  $(\text{NH}_4)_2\text{SO}_4$ , 4 mM  $\text{MgSO}_4$ , 17 mM sodium citrate, 132 mM  $\text{KH}_2\text{PO}_4$ , 36 mM  $\text{K}_2\text{HPO}_4$  was prepared with molecular pure water and filter-sterilized. Freezing medium was prepared by diluting the 10× freezing medium with the LB medium. An aliquot (200 µl) of the freezing medium was used for each well of the 96-well archive plates (cat #3370, Corning Inc. Corning, N.Y.).

**[0181]** Clones from the LB agar plates were selected and inoculated into the 96-well archive plates containing the freezing medium and grown overnight at 37° C. without shaking. The archive plates were then stored at -80° C. *E. coli* strain Bw25113 transformed with pBAD-HisB (Invitrogen) was always used as the negative control. For libraries C, E, F and G, mutant T52D of (PF5-ilvC) was used as the positive control. The mutant T52D was a mutant of PF5-ilvC in which

the threonine at position 52 was changed to aspartic acid. For library H, mutant C3B11 (R47F/S50A/T52D/v53W of PF5-ilvC) was used as the positive control.

**[0182]** Clones from archive plates were inoculated into the 96-deep well plates. Each well contained 3.0 µl of cells from thawed archive plates, 300 µl of the LB medium containing 100 µg/ml ampicillin and 0.02% (w/v) arabinose as the inducer. Cells were grown overnight at 37° C. with 80% humidity while shaking (900 rpm), harvested by centrifugation (4000 rpm, 5 min at 25° C.). (Eppendorf centrifuge, Brinkmann Instruments, Inc. Westbury, N.Y.) and the cell pellet was stored at -20° C. for later analysis.

**[0183]** The assay substrate, (R,S)-acetolactate, was synthesized as described by Aulabaugh and Schloss (Aulabaugh and Schloss, *Biochemistry*, 29, 2824-2830, 1990): 1.0 g of 2-acetoxy-2-methyl-3-oxobutyric acid ethyl ester (Aldrich, Milwaukee, Wis.) was mixed with 10 ml NaOH (1.0 M) and stirred at room temperature. When the solution's pH became neutral, additional NaOH was slowly added until pH~8.0 was maintained. All other chemicals used in the assay were purchased from Sigma.

**[0184]** The enzymatic conversion of acetolactate to α-dihydroxy-isovalerate by KARI was followed by measuring the disappearance of the cofactor, NADPH or NADH, from the reaction at 340 nm using a plate reader (Molecular Device, Sunnyvale, Calif.). The activity was calculated using the molar extinction coefficient of 6220 M cm<sup>-1</sup> for either NADPH or NADH. The stock solutions used were:  $\text{K}_2\text{HPO}_4$  (0.2 M);  $\text{KH}_2\text{PO}_4$  (0.2 M); EDTA (0.5 M);  $\text{MgCl}_2$  (1.0 M); NADPH (2.0 mM); NADH (2.0 mM) and acetolactate (45 mM). The 100 ml reaction buffer mix stock containing: 4.8 ml  $\text{K}_2\text{HPO}_4$ , 0.2 ml  $\text{KH}_2\text{PO}_4$ , 4.0 ml  $\text{MgCl}_2$ , 0.1 ml EDTA and 90.9 ml water was prepared.

**[0185]** Frozen cell pellet in deep-well plates and BugBuster were warmed up at room temperature for 30 min at the same time. Each well of 96-well assay plates was filled with 120 µl of the reaction buffer and 20 µl of NADH (2.0 mM), 150 µl of BugBuster was added to each well after 30 min warm-up and cells were suspended using Genmate (Tecan Systems Inc. San Jose, Calif.) by pipetting the cell suspension up and down (x5). The plates were incubated at room temperature for 20 min and then heated at 60° C. for 10 min. The cell debris and protein precipitates were removed by centrifugation at 4,000 rpm for 5 min at 25° C. An aliquot (50 µl) of the supernatant was transferred into each well of 96-well assay plates, the solution was mixed and the bubbles were removed by centrifugation at 4000 rpm at 25° C. for 1 min. Absorbance at 340 nm was recorded as background, 20 µl of acetolactate (4.5 mM, diluted with the reaction buffer) was added to each well and mixed with shaking by the plate reader. Absorbance at 340 nm was recorded at 0, and 60 minutes after substrate addition. The difference in absorbance (before and after substrate addition) was used to determine the activity of the mutants. Mutants with higher KARI activity compared to the wild type were selected for re-screening.

**[0186]** About 5,000 clones were screened for library C and 360 top performers were selected for re-screen. About 92 clones were screened for library E and 16 top performers were selected for re-screening. About 92 clones were screened for library F and 8 top performers were selected for re-screening. About 92 clones were screened for library G and 20 top performers were selected for re-screening. About 8,000 clones were screened for library H and 62 top performers were selected for re-screening.

**[0187]** For library C, about 360 top performers were re-screened using the same procedure as for the general screening. Among them, 45 top performers were further selected for re-screening as described below.

#### Secondary Assay of Active Mutants

**[0188]** Cells containing pBad-ilvC and its mutants identified by high throughput screening were grown overnight, at 37° C., in 3.0 ml of the LB medium containing 100 µg/ml ampicillin and 0.02% (w/v) arabinose as the inducer while shaking at 250 rpm. The cells were then harvested by centrifugation at 18,000×g for 1 min at room temperature (Sigma micro-centrifuge model 1-15, Laurel, Md.). The cell pellets were re-suspended in 300 µl of BugBuster Master Mix (EMD Chemicals). The reaction mixture was first incubated at room temperature for 20 min and then heated at 60° C. for 10 min. The cell debris and protein precipitate were removed by centrifugation at 18,000×g for 5 min at room temperature.

**[0189]** The reaction buffer (120 µl) prepared as described above was mixed with either NADH or NADPH (20 µl) stock and cell extract (20 µl) in each well of a 96-well assay plate. The absorbance at 340 nm at 25° C. was recorded as background. Then 20 µl of acetolactate (4.5 mM, diluted with reaction buffer) was added each well and mixed with shaking by the plate reader. The absorbance at 340 nm at 0 min, 2 min and 5 min after adding acetolactate was recorded. The absorbance difference before and after adding substrate was used to determine the activity of the mutants. The mutants with high activity were selected for sequencing.

**[0190]** Five top performers from "Library C" were identified and sequenced (FIG. 4). The best performer was mutant R47F/S50A/T52D/V53W, which completely reversed the nucleotide specificity. The best performers from "Libraries E, F and G" were R47P, S50D and T52D respectively (FIG. 5). For "Library H", 5 top performers were identified and sequenced (FIG. 6) and the best performer was R47P/S50G/T52D, which also completely reversed the nucleotide specificity. Enzymes containing activities higher than the background were considered positive.

#### Kari Enzyme Assay

**[0191]** KARI enzyme activity can be routinely measured by NADH or NADPH oxidation as described above, however to measure formation of the 2,3-dihydroxyisovalerate product directly, analysis of the reaction was performed using LC/MS.

**[0192]** Protein concentration of crude cell extract from Bugbuster lysed cells (as described above) was measured using the BioRad protein assay reagent (BioRad Laboratories, Inc., Hercules, Calif. 94547). A total of 0.5 micrograms of crude extract protein was added to a reaction buffer consisting of 100 mM HEPES-KOH, pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM glucose-6-phosphate (Sigma-Aldrich), 0.2 Units of *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase (Sigma-Aldrich), and various concentrations of NADH or NADPH, to a volume of 96 µL. The reaction was initiated by the addition of 4 µL of acetolactate to a final concentration of 4 mM and a final volume of 100 µL. After timed incubations at 30° C., typically between 2 and 15 min, the reaction was quenched by the addition of 10 µL of 0.5 M EDTA pH 8.0 (Life Technologies, Grand Island, N.Y. 14072). To measure the K<sub>M</sub> of NADH, the concentrations used were 0.03, 0.1, 0.3, 1, 3, and 10 mM.

**[0193]** To analyze for 2,3-dihydroxyisovalerate, the sample was diluted 10× with water, and 8.0 µl was injected into a Waters Acquity HPLC equipped with Waters SQD mass spectrometer (Waters Corporation, Milford, Mass.). The chromatography conditions were: flow rate (0.5 ml/min), on a Waters Acquity HSS T3 column (2.1 mm diameter, 100 mm length). Buffer A consisted of 0.1% (v/v) in water, Buffer B was 0.1% formic acid in acetonitrile. The sample was analyzed using 1% buffer B (in buffer A) for 1 min, followed by a linear gradient from 1% buffer B at 1 min to 75% buffer B at 1.5 min. The reaction product, 2,3-dihydroxyisovalerate, was detected by ionization at m/z=133, using the electrospray ionization device at -30 V cone voltage. The amount of product 2,3-dihydroxyisovalerate was calculated by comparison to an authentic standard.

**[0194]** To calculate the K<sub>M</sub> for NADH, the rate data for DHIV formation was plotted in Kaleidagraph (Synergy Software, Reading, Pa.) and fitted to the single substrate Michaelis-Menton equation, assuming saturating acetolactate concentration.

#### Example 1

##### Construction of Site-Saturation Gene Libraries

**[0195]** Seven gene libraries were constructed (Table 2) using two steps: 1) synthesis of MegaPrimers using commercially synthesized oligonucleotides described in Table 1; and 2) construction of mutated genes using the MegaPrimers obtained in step 1. These primers were prepared using high fidelity pfu-ultra polymerase (Stratagene, La Jolla, Calif.) for one pair of primer containing one forward and one reverse primer. The templates for libraries C, E, F, G and H were the wild type of PF5\_ilvC. The DNA templates for library N were those mutants having detectable NADH activity from library C while those for library O were those mutants having detectable NADH activity from library H. A 50 µl reaction mixture contained: 5.0 µl of 10× reaction buffer supplied with the pfu-ultra polymerase (Stratagene), 1.0 µl of 50 ng/µl template, 1.0 µl each of 10 pmol/µl forward and reverse primers, 1.0 µl of 40 mM dNTP mix (Promega, Madison, Wis.), 1.0 µl pfu-ultra DNA polymerase (Stratagene) and 39 µl water. The mixture was placed in a thin well 200 µl tube for the PCR reaction in a Mastercycler gradient equipment (Brinkmann Instruments, Inc. Westbury, N.Y.). The following conditions were used for the PCR reaction: The starting temperature was 95° C. for 30 sec followed by 30 heating/cooling cycles. Each cycle consisted of 95° C. for 30 sec, 54° C. for 1 min, and 70° C. for 2 min. At the completion of the temperature cycling, the samples were kept at 70° C. for 4 min more, and then held awaiting sample recovery at 4° C. The PCR product was cleaned up using a DNA cleaning kit (Cat#D4003, Zymo Research, Orange, Calif.) as recommended by the manufacturer.

TABLE 2

GENE LIBRARIES			
Library name	Templates	Targeted position(s) of PF5_ilvC	Primers used
C	PF5_ilvC	47, 50, 52 and 53	SEQ ID No: 1 and 2
E	PF5_ilvC	47	SEQ ID No: 1 and 3
F	PF5_ilvC	50	SEQ ID No: 1 and 4
G	PF5_ilvC	52	SEQ ID No: 1 and 5

TABLE 2-continued

GENE LIBRARIES			
Library name	Templates	Targeted position(s) of Pf5_ilvC	Primers used
H	Pf5_ilvc	47, 50, and 52	SEQ ID No: 1 and 6
N	Good mutants from library C	53	SEQ ID NO: 20 and 21
O	Good mutants from library H	53	SEQ ID NO: 20 and 21

[0196] The MegaPrimers were then used to generate gene libraries using the QuickChange II XL site directed mutagenesis kit (Catalog #200524, Stratagene, La Jolla Calif.). A 50  $\mu$ l reaction mixture contained: 5.0  $\mu$ l of 10 $\times$  reaction buffer, 1.0  $\mu$ l of 50 ng/ $\mu$ l template, 42  $\mu$ l Megaprimer, 1.0  $\mu$ l of 40 mM dNTP mix, 1.0  $\mu$ l pfu-ultra DNA polymerase. Except for the MegaPrimer and the templates, all reagents used here were supplied with the kit indicated above. This reaction mixture was placed in a thin well 200  $\mu$ l-capacity PCR tube and the following reactions were used for the PCR: The starting temperature was 95 $^{\circ}$  C. for 30 sec followed by 25 heating/cooling cycles. Each cycle consisted of 95 $^{\circ}$  C. for 30 sec, 55 $^{\circ}$  C. for 1 min, and 68 $^{\circ}$  C. for 6 min. At the completion of the temperature cycling, the samples were kept at 68 $^{\circ}$  C. for 8 min more, and then held at 4 $^{\circ}$  C. for later processing. Dpn I restriction enzyme (1.0  $\mu$ l) (supplied with the kit above) was directly added to the finished reaction mixture, enzyme digestion was performed at 37 $^{\circ}$  C. for 1 hr and the PCR product was cleaned up using a DNA cleaning kit (Zymo Research). The cleaned PCR product (10  $\mu$ l) contained mutated genes for a gene library.

[0197] The cleaned PCR product was transformed into an electro-competent strain of *E. coli* Bw25113 ( $\Delta$ ilvC) using a BioRad Gene Pulser II (Bio-Rad Laboratories Inc., Hercules, Calif.). The transformed clones were streaked on agar plates containing the Lauria Broth medium and 100  $\mu$ g/ml ampicillin (Cat#L1004, Teknova Inc. Hollister, Calif.) and incubated at 37 $^{\circ}$  C. overnight. Dozens of clones were randomly chosen for DNA sequencing to confirm the quality of the library.

TABLE 3

List of some mutants having NADH activities identified from saturation libraries				
Mutant	Position 47	Position 50	Position 52	Position 53
SD2	R47Y	S50A	T52H	V53W
SB1	R47Y	S50A	T52G	V53W
SE1	R47A	S50W	T52G	V53W
SH2	R47N	S50W	T52N	V53W
SB2	R47I		T52G	V53W
SG1	R47Y		T52G	V53W
SB3	R47G	S50W	T52G	V53W
SE2	R47P	S50E	T52A	V53W
SD3	R47L	S50W	T52G	V53W
C2A6	R47I	S50G	T52D	V53W
C3E11	R47A	S50M	T52D	V53W
C3A7	R47Y	S50A	T52D	V53W
C3B11	R47F	S50A	T52D	V53W
C4A5	R47Y	S50A	T52S	V53W
C3B12	R47I		T52D	V53W
C4H7	R47I		T52S	V53W
C1D3	R47G	S50M	T52D	V53W
C4D12	R47C	S50W	T52G	V53W
C1G7	R47P	S50G	T52D	V53W

TABLE 3-continued

List of some mutants having NADH activities identified from saturation libraries				
Mutant	Position 47	Position 50	Position 52	Position 53
C2F6	R47P	S50V	T52D	V53W
C1C4	R47P	S50E	T52S	V53W
6924F9	R47P	S50G	T52D	
6881E11	R47P	S50N	T52C	
6868F10	R47P		T52S	
6883G10	R47P	S50D	T52S	
6939G4	R47P	S50C	T52D	
11463D8	R47P	S50F	T52D	
9667A11	R47N	S50N	T52D	V53A
9675C8	R47Y	S50A	T52D	V53A
9650E5	R47N	S50W	T52G	V53H
9875B9	R47N	S50N	T52D	V53W
9862B9	R47D	S50W	T52G	V53W
9728G11	R47N	S50W	T52G	V53W
11461D8	R47F	S50A	T52D	V53A
11461A2	R47P	S50F	T52D	V53I

## Example 2

## Construction of Error Prone PCR Libraries

[0198] Several rounds of error prone PCR (epPCR) libraries were created using the GeneMorph II kit (Stratagene) as recommended by the manufacturer. All the epPCR libraries target the N-terminal of PF5—KARI. The forward primer (SED ID No: 20) and the reverse primer (SED ID No: 22) were used for all epPCR libraries.

[0199] The DNA templates for each epPCR library were mutants having relatively good NADH activities from the previous library. For example: the DNA templates for the n<sup>th</sup> epPCR library were mutants having good NADH activities from the (n-1)th epPCR library. The templates of the first epPCR library were mutants having relatively good NADH activities from libraries N and O. The mutations rate of template made by this kit was controlled by the amount of template added in the reaction mixture and the number of amplification cycles. Typically, 1.0 ng of each DNA template was used in 100  $\mu$ l of reaction mixture. The number of amplification cycles was 70. The following conditions were used for the PCR reaction: The starting temperature was 95 $^{\circ}$  C. for 30 sec followed by 70 heating/cooling cycles. Each cycle consisted of 95 $^{\circ}$  C. for 30 sec, 55 $^{\circ}$  C. for 30 min, and 70 $^{\circ}$  C. for 2 min. After the first 35 heating/cooling cycles finished, more dNTP and Mutazyme II DNA polymerase were added. The PCR product was cleaned up using a DNA cleaning kit (Cat#D4003, Zymo Research, Orange, Calif.) as recommended by the manufacturer. The cleaned PCR product was treated as megaprimer and introduced into the vector using the Quickchange kit as described in Example 1. Table 4 below lists the KARI mutants obtained and the significant improvement observed in their binding NADH. The K<sub>m</sub> was reduced from 1100  $\mu$ M for mutant C3B11 to 50  $\mu$ M for mutant 12957G9.

TABLE 4

List of some mutants with their measured $K_m$ values		
Mutant	Mutation Locations	NADH $K_m$ ( $\mu$ M)
C3B11	R47F/S50A/T52D/V53W	1100
SB3	R47G/S50W/T52G/V53W	500
11518B4	R47N/S50N/T52D/V53A/A156V	141
11281G2	R47N/S50N/T52D/V53A/A156V/L165M	130
12985F6	R47Y/S50A/T52D/V53A/L61F/A156V	100
13002D8	R47Y/S50A/T52D/V53A/L61F/A156V/G170A	68
12957G9	Y24F/R47Y/S50A/T52D/V53A/L61F/G170A	50
12978D9	R47Y/S50A/T52D/V53A/L61F/Q115L/A156V	114

## Example 3

## Thermostability of PF5-ilvC and its Mutants

[0200] Cells containing mutated pBad-ilvC were grown overnight at 37° C. in 25 ml of the LB medium containing 100  $\mu$ g/ml ampicillin and 0.02% (w/v) arabinose inducer while shaking at 250 rpm. The cells were then harvested by centrifugation at 18,000 $\times$ g for 1 min at room temperature and the cell pellets were re-suspended in 300  $\mu$ l of BugBuster Master Mix (EMD Chemicals). The reaction mixture was first incubated at room temperature for 20 min and aliquots of this cell mixture (e.g. 50  $\mu$ l) were incubated at different temperatures (from room temperature to 75° C.) for 10 min. The precipitate was removed by centrifugation at 18,000 $\times$ g for 5 min at room temperature. The remaining activity of the supernatant was analyzed as described above. As shown in FIG. 7, pBad-ilvC was very stable with  $T_{50}$  equal to 68° C. ( $T_{50}$  is the temperature, at which 50% of protein lost its activity after 10 min incubation).

[0201] The thermostability of PF5-ilvC allowed destruction of most of the other non-KARI NADH oxidation activity within these cells, reducing the NADH background consumption and thus facilitating the KARI activity assays. This heat treatment protocol was used in all screening and re-screening assays. The mutants thus obtained were all thermostable which allowed easier selection of the desirable mutants.

## Example 4

## Stoichiometric Production of 2,3-Dihydroxyisovalerate by Kari During Consumption of Cofactors NADH or NADPH

[0202] Screening and routine assays of KARI activity rely on the 340 nm absorption decrease associated with oxidation of the pyridine nucleotides NADPH or NADH. To insure that this metric was coupled to formation of the other reaction product, oxidation of pyridine nucleotide and formation of 2,3-dihydroxyisovalerate were measured in the same samples.

[0203] The oxidation of NADH or NADPH was measured at 340 nm in a 1 cm path length cuvette on a Agilent model 8453 spectrophotometer (Agilent Technologies, Wilmington Del.). Crude cell extract (0.1 ml) prepared as described above containing either wild type PF5 KARI or the C3B11 mutant, was added to 0.9 ml of K-phosphate buffer (10 mM, pH 7.6), containing 10 mM  $MgCl_2$ , and 0.2 mM of either NADPH or NADH. The reaction was initiated by the addition of acetolactate to a final concentration of 0.4 mM. After 10-20% decrease in the absorption (about 5 min), 50  $\mu$ l of the reaction

mixture was rapidly withdrawn and added to a 1.5 ml Eppendorf tube containing 10  $\mu$ l 0.5 mM EDTA to stop the reaction and the actual absorption decrease for each sample was accurately recorded. Production of 2,3-dihydroxyisovalerate was measured and quantitated by LC/MS as described above.

[0204] The coupling ratio is defined by the ratio between the amount of 2,3-dihydroxyisovalerate (DHIV) produced and the amount of either NADH or NADPH consumed during the experiment. The coupling ratio for the wild type enzyme (PF5-ilvC), using NADPH, was 0.98 DHIV/NADPH, while that for the mutant (C3B11), using NADH, was on average around 1.10 DHIV/NADPH.

## Example 5

## Identification of Amino Acids Involved in Cofactor Binding in Kari for Cofactor Specificity Switching Using Bioinformatic Tools

[0205] To discover if naturally existing KARI sequences could provide clues for amino acid positions that should be targeted for mutagenesis, multiple sequence alignment (MSA) using PF5\_KARI, its close homolog PAO1\_KARI and three KARI sequences with measurable NADH activity, i.e., *B. Cereus* ilvC1 and ilvC2 and spinach KARI were performed (FIG. 8). Based on the multiple sequence alignment, positions 33, 43, 59, 61, 71, 80, 101, and 119 were chosen for saturation mutagenesis. Saturation mutagenesis on all of these positions was performed simultaneously using the QuickChange II XL site directed mutagenesis kit (Catalog #200524, Stratagene, La Jolla Calif.) with the manufacturer's suggested protocol. Starting material for this mutagenesis was a mixed template consisting of the mutants already identified in Table 4 and Example 2. The primers used are listed in Table 5. The library of mutants thus obtained were named library Z". Mutants with good NADH activity from this library were identified using high throughput screening and their KARI activity and the  $K_m$  for NADH were measured as described above. These mutants (listed in Table 6) possess much lower  $K_m$ s for NADH compared to the parent templates (Table 4). A megaprimer, using primers (SEQ ID Nos. 20 and 58), was created and mutations at positions 156 and 170 were eliminated. Further screening of this set of mutants identified mutant 3361 G8 (SEQ ID NO: 67)(Table 7). The hits from library Z were further subjected to saturation mutagenesis at position 53 using primers (SEQ ID Nos. 20 and 21), and subsequent screening identified the remaining mutants in Table 7. As shown in Table 7 the new mutants possessed much lower  $K_m$  for NADH (e.g., 4.0 to 5.5  $\mu$ M) compared to mutants listed in Table 6 (e.g., 14-40 M).

TABLE 5

Primers for Example 5	
Targeted position(s) of Pf5_ilvC	Primers
33	pBAD-405-C33_090808f : GCTCAAGCANNKACCTGAAGG (SEQ ID NO: 49) pBAD-427-C33_090808r : CCTTCAGGTTKNNNGCTTGAGC (SEQ ID NO: 50)
43	pBAD-435-T43_090808f : GTAGACGTGNKGTGGCCTG

TABLE 5-continued

Primers for Example 5	
Targeted position(s) of Pf5_ilvC	Primers
	(SEQ ID NO: 51) pBAD-456-T43_090808r: CAGGCCAACKNNACGCTCTAC (SEQ ID NO: 52)
59 and 61	pBAD-484-H59L61_090808f: CTGAAGCCNNKGGCINNKAAGTGAC (SEQ ID NO: 53) pBAD-509-H59L61_090808r: GTCACCTTKNNGCCKNNGGCTTCAG (SEQ ID NO: 54)
71	pBAD-519-A71_090808f: GCAGCCGTTNNKGGTGCCGACT (SEQ ID NO: 55) pBAD-541-A71_090808r: AGTCGGCACCKNNAACGGCTGC (SEQ ID NO: 56)
80	pBAD-545-T80_090808f: CATGATCCTGNNKCCGGACGAG (SEQ ID NO: 57) pBAD-567-T80_090808r: CTCGTCCGGKNNCAGGATCATG (SEQ ID NO: 58)
101	pBAD-608-A101_090808f: CAAGAAGGGCNNKACTCTGGCCT (SEQ ID NO: 59) pBAD-631-A101_090808r: AGGCCAGAGTKNNGCCCTTCTTG (SEQ ID NO: 60)
119	pBAD-663-R119_090808f: GTTGTGCCTNNKCCGACCTCG (SEQ ID NO: 61) pBAD-685-R119_090808r: CGAGGTCGGCKNNAGGCACAAC (SEQ ID NO: 62)

TABLE 6

List of some mutants with their measured $K_m$ values (the mutated positions in those mutants were identified by bioinformatic tools)		
Mutant	Mutation Locations	NADH $K_m$ ( $\mu$ M)
ZB1	Y24F/R47Y/S50A/T52D/V53A/L61F/A156V (SEQ ID NO: 24)	40
ZF3	Y24F/C33L/R47Y/S50A/T52D/V53A/L61F (SEQ ID NO: 25)	21
ZF2	Y24F/C33L/R47Y/S50A/T52D/V53A/L61F/A156V (SEQ ID NO: 26)	17
ZB3	Y24F/C33L/R47Y/S50A/T52D/V53A/L61F/G170A (SEQ ID NO: 27)	17
Z4B8	C33L/R47Y/S50A/T52D/V53A/L61F/T80I/A156V (SEQ ID NO: 28)	14

TABLE 7

Mutants further optimized for improved $K_m$ (for NADH)		
Mutant	Mutation Locations	NADH $K_m$ ( $\mu$ M)
3361G8	C33L/R47Y/S50A/T52D/V53A/L61F/T80I (SEQ ID NO: 67)	5.5
2H10	Y24F/C33L/R47Y/S50A/T52D/V53L61F/T80I/ A156V (SEQ ID NO: 68)	5.3
1D2	Y24F/R47Y/S50A/T52D/V53A/L61F/T80I/A156V (SEQ ID NO: 69)	4.1
3F12	Y24F/C33L/R47Y/S50A/T52D/V53A/L61F/T80I/ A156V (SEQ ID NO: 70)	4.0

[0206] Further analyses using bioinformatic tools were therefore performed to expand the mutational sites to other KARI sequences as described below.

#### Sequence Analysis

[0207] Members of the protein family of ketol-acid reductoisomerase (KARI) were identified through BlastP searches of publicly available databases using amino acid sequence of *Pseudomonas fluorescens* PF5 KARI (SEQ ID NO:17) with the following search parameters: E value=10, word size=3, Matrix=Blosum62, and Gap opening=11 and gap extension=1, E value cutoff of  $10^{-3}$ . Identical sequences and sequences that were shorter than 260 amino acids were removed. In addition, sequences that lack the typical GxGXX (G/A) motif involved in the binding of NAD(P)H in the N-terminal domain were also removed. These analyses resulted in a set of 692 KARI sequences.

[0208] A profile HMM was generated from the set of the experimentally verified Class I and Class II KARI enzymes from various sources as described in Table 8. Details on building, calibrating, and searching with this profile HMM are provided below. Any sequence that can be retrieved by HMM search using the profile HMM for KARI at E-value above  $1E^{-3}$  is considered a member of the KARI family. Positions in a KARI sequence aligned to the following in the profile HMM nodes (defined below in the section of profile HMM building) are claimed to be responsible for NADH utilization: 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, and 170 (the numbering is based on the sequences of *Pseudomonas fluorescens* PF5 KARI).

#### Preparation of Profile HMM

[0209] A group of KARI sequences were expressed in *E. coli* and have been verified to have KARI activity. These KARIs are listed in Table 6. The amino acid sequences of these experimentally verified functional KARIs were analyzed 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 (profile HMM) that characterizes the input sequences. As stated in the user guide, profile HMMs are statistical descriptions of the consensus of a multiple sequence alignment. They use position-specific scores for amino acids (or nucleotides) and position specific

scores for opening and extending an insertion or deletion. Compared to other profile based methods, HMMs have a formal probabilistic basis. Profile HMMs for a large number of protein families are publicly available in the PFAM database (Janelia Farm Research Campus, Ashburn, Va.).

**[0210]** The profile HMM was built as follows:

#### Step 1. Build a Sequence Alignment

**[0211]** The 25 sequences for the functionally verified KARIs listed above were aligned using Clustal W (Thompson, J. D., Higgins, D. G., and Gibson T. J. (1994) *Nuc. Acid Res.* 22: 46734680) with default parameters. The alignment is shown in FIG. 9.

will be assigned as an insert column. By default,  $x$  is set to 0.5. Each match state has an I and a D state associated with it. HMMER calls a group of three states (M/D/I) at the same consensus position in the alignment a “node”.

**[0214]** A profile HMM has several types of probabilities associated with it. One type is the transition probability—the probability of transitioning from one state to another. There are also emissions probabilities associated with each match state, based on the probability of a given residue existing at that position in the alignment. For example, for a fairly well-conserved column in an alignment, the emissions probability for the most common amino acid may be 0.81, while for each of the other 19 amino acids it may be 0.01.

TABLE 8

25 Experimentally verified KARI enzymes			
GI Number	Accession	SEQ ID NO:	Organism
70732562	YP_262325.1	17	<i>Pseudomonas fluorescens</i> Pf-5
15897495	NP_342100.1	13	<i>Sulfolobus solfataricus</i> P2
18313972	NP_560639.1	14	<i>Pyrobaculum aerophilum</i> str. IM2
76801743	YP_326751.1	30	<i>Natronomonas pharaonis</i> DSM 2160
16079881	NP_390707.1	31	<i>Bacillus subtilis</i> subsp. <i>subtilis</i> str. 168
19552493	NP_600495.1	32	<i>Corynebacterium glutamicum</i> ATCC 13032
6225553	O32414	33	<i>Phaeosporidium molischianum</i>
17546794	NP_520196.1	15	<i>Ralstonia solanacearum</i> GM11000
56552037	YP_162876.1	34	<i>Zymomonas mobilis</i> subsp. <i>mobilis</i> ZM4
114319705	YP_741388.1	35	<i>Alkalilimnicola ehrlichei</i> MLHE-1
57240359	ZP_00368308.1	36	<i>Campylobacter lari</i> RM2100
120553816	YP_958167.1	37	<i>Marinobacter aquaeolei</i> VT8
71065099	YP_263826.1	38	<i>Psychrobacter arcticus</i> 273-4
83648555	YP_436990.1	39	<i>Hahella chejuensis</i> KCTC 2396
74318007	YP_315747.1	40	<i>Thiobacillus denitrificans</i> ATCC 25259
67159493	ZP_00420011.1	41	<i>Azotobacter vinelandii</i> AvOP
66044103	YP_233944.1	42	<i>Pseudomonas syringae</i> pv. <i>syringae</i> B728a
28868203	NP_790822.1	43	<i>Pseudomonas syringae</i> pv. <i>tomato</i> str. DC3000
26991362	NP_746787.1	44	<i>Pseudomonas putida</i> KT2440
104783656	YP_610154.1	45	<i>Pseudomonas entomophila</i> L48
146306044	YP_001186509.1	46	<i>Pseudomonas mendocina</i> ymp
15599888	NP_253382.1	16	<i>Pseudomonas aeruginosa</i> PAO1
42780593	NP_977840.1	47	<i>Bacillus cereus</i> ATCC 10987
42781005	NP_978252.1	48	<i>Bacillus cereus</i> ATCC 10987
266346	Q01292	18	<i>Spinacia oleracea</i>

#### Step 2. Build a Profile HMM

**[0212]** The hmmbuild program was run on the set of aligned sequences using default parameters. hmmbuild reads the multiple sequence alignment file, builds a new profile HMM, and saves the profile HMM to file. Using this program an un-calibrated profile was generated from the multiple sequence alignment for twenty-four experimentally verified KARIs as described above.

**[0213]** The following information based on the HMMER software user guide gives some description of the way that the hmmbuild program prepares a profile HMM. A profile HMM is a linear state machine consisting of a series of nodes, each of which corresponds roughly to a position (column) in the multiple sequence alignment from which it is built. If gaps are ignored, the correspondence is exact, i.e., the profile HMM has a node for each column in the alignment, and each node can exist in one state, a match state. The word “match” here implies that there is a position in the model for every position in the sequence to be aligned to the model. Gaps are modeled using insertion (I) states and deletion (D) states. All columns that contain more than a certain fraction  $x$  of gap characters

**[0215]** A profile HMM is completely described in a HMMER2 profile save file, which contains all the probabilities that are used to parameterize the HMM. The emission probabilities of a match state or an insert state are stored as log-odds ratio relative to a null model:  $\log_2(p_x)/(\text{null}_x)$ . Where  $p_x$  is the probability of an amino acid residue, at a particular position in the alignment, according to the profile HMM and  $\text{null}_x$  is the probability according to the Null model. The Null model is a simple one state probabilistic model with pre-calculated set of emission probabilities for each of the 20 amino acids derived from the distribution of amino acids in the SWISSPROT release 24. State transition scores are also stored as log odds parameters and are proportional to  $\log_2(t_x)$ . Where  $t_x$  is the transition probability of transitioning from one state to another state.

#### Step 3. Calibrate the Profile HMM

**[0216]** The profile HMM was read using hmmscalibrate which scores a large number of synthesized random sequences with the profile (the default number of synthetic sequences used is 5,000), fits an extreme value distribution



(EVD) to the histogram of those scores, and re-saves the HMM file now including the EVD parameters. These EVD parameters ( $\mu$  and  $\lambda$ ) are used to calculate the E-values of bit scores when the profile is searched against a protein sequence database. Hmcalibrate writes two parameters into the HMM file on a line labeled "EVD": these parameters are the  $p$  (location) and  $\lambda$  (scale) parameters of an extreme value distribution (EVD) that best fits a histogram of scores calculated on randomly generated sequences of about the same length and residue composition as SWISS-PROT. This calibration was done once for the profile HMM.

[0217] The calibrated profile HMM for the set of KARI sequences is provided appended hereto as a profile HMM Excel chart (Table 9). In the main model section starting from the HMM flag line, the model has three lines per node, for M nodes (where M is the number of match states, as given by the LENG line). The first line reports the match emission log-odds scores: the log-odds ratio of emitting each amino acid from that state and from the Null model. The first number is the node number (1.M). The next K numbers for match emission scores, one per amino acid. The highest scoring amino acid is indicated in the parenthesis after the node number. These log-odds scores can be converted back to HMM probabilities using the null model probability. The last number on the line represents the alignment column index for this match state. The second line reports the insert emission scores, and the third line reports on state transition scores: M→M, M→I, M→D; I→M, I→I; D→M, D→D; B→M; M→E.

Step 4. Test the Specificity and Sensitivity of the Built Profile HMMs

[0218] The Profile HMM was evaluated using hmmsearch, which reads a Profile HMM from hmmfile and searches a sequence file for significantly similar sequence matches. The sequence file searched contained 692 sequences (see above). During the search, the size of the database (Z parameter) was set to 1 billion. This size setting ensures that significant E-values against the current database will remain significant in the foreseeable future. The E-value cutoff was set at 10.

[0219] A hmmer search, using hmmsearch, with the profile HMM generated from the alignment of the twenty-five

KARIs with experimentally verified function, matched all 692 sequences with an E value <  $10^{-3}$ . This result indicates that members of the KARI family share significant sequence similarity. A hmmer search with a cutoff of E value  $10^{-3}$  was used to separate KARIs from other proteins.

Step 5. Identify Positions that are Relevant for NAD(P)H Utilization.

[0220] Eleven positions have been identified in KARI of *Pseudomonas fluorescens* Pf-5 that switches the cofactor from NADPH to NADH. Since the KARI sequences share significant sequence similarity (as described above), it can be reasoned that the homologous positions in the alignment of KARI sequences should contribute to the same functional specificity. The profile HMM for KARI enzymes has been generated from the multiple sequence alignment which contains the sequence of *Pseudomonas fluorescens* Pf-5 KARI. The eleven positions in the profile HMM representing the columns in the alignment which correspond to the eleven cofactor switching positions in *Pseudomonas fluorescens* Pf-5 KARI are identified as positions 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, and 170. The lines corresponding to these positions in the model file are highlighted in yellow in Table 9.

[0221] For any query sequence, hmm search is used to search the profile HMM for KARI against the query sequence and the alignment of the query to the HMM is recorded in the output file. In the alignment section of the output, the top line is the HMM consensus. The amino acid shown for the consensus is the highest probability amino acid at that position according to the HMM (not necessarily the highest scoring amino acid). The center line shows letters for "exact" matches to the highest probability residue in the HMM, or a "+" when the match has a positive score. The third line shows the sequence itself. The positions in the query sequence that are deemed as relevant for cofactor switching are identified as those that are aligned to these eleven nodes in the profile HMM as described above. An example of the alignment of *Pseudomonas fluorescens* Pf-5 KARI to the profile HMM of KARI is shown in FIG. 10 and the eleven positions that are responsible for cofactor switching are shaded in grey.

TABLE 9

HMM	A m->m	C m->i	D m->d	E i->m	F i->i	G d->m	H d->d	I b->m	K m->e	L	M	N
	-650	*	-1463									
1(Q)	-648	-1356	-136	-44	-1453	-1166	-219	-1455	321	-1417	-911	-227
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-701	-1378	-650	*			
2(M)	-4231	-3929	-5216	-5402	-3438	-4370	-4528	-3232	-5113	-2613	5320	-5052
—	-147	-501	232	42	-382	397	104	-625	209	-467	-722	276
—	-3303	-3318	-325	-3473	-136	-701	-1378	*	*			
3(F)	-1308	-1104	-2227	-2120	3516	-2093	-244	-196	-1891	64	66	-1626
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-943	-1060	*	*			
4(A)	1616	-1744	1125	33	-2015	-1540	-262	-1686	937	-1765	-911	-252
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-901	-7402	-1125	-894	-1115	-2352	-314	*	*			
5(C)	-346	2578	1084	-712	2092	-1540	-384	-167	-624	-482	125	-731
—	-149	-500	235	43	-381	398	106	-626	210	-466	-721	275
—	-1009	-1006	-7567	-131	-3527	-1916	-444	*	*			
6(S)	800	-586	-1937	-1415	-821	-1740	-954	1279	-1204	-584	19	-1258
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-17	-6953	-7995	-894	-1115	-146	-3378	*	*			

TABLE 9-continued

7(K)	-956	-2411	-803	501	-2743	-1919	-558	-2483	2435	-2420	-1502	57
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
8(V)	-2472	-2010	-5089	-4702	-2534	-4789	-4391	2241	-4574	-151	-1318	-4442
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
9(Y)	-4673	-3685	-5210	-5505	2423	-5069	-1332	-3424	-5065	-392	-2838	-3726
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
10(Y)	-2170	-2625	-2489	-2097	-1555	-2986	-1481	-2628	906	-2674	-2098	-2051
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
11(D)	-2498	-4412	3500	1042	-4581	-2437	-1765	-4500	733	-4361	-3682	515
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
12(K)	11	-2371	348	819	-2692	-535	-527	-2443	2294	-2387	-1461	590
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
13(D)	-2663	-4633	3700	580	-4789	-2487	-1872	-4738	731	-4578	-3963	-1073
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
14(C)	2503	3193	-4266	-3818	-2010	-3276	-2896	762	-3517	-1437	-1051	-3233
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
15(D)	-1363	-2905	2748	542	-3202	-2072	-920	-2977	290	-2912	-2023	1270
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
16(L)	-1268	-1113	-3338	-540	-1057	-2827	-1716	569	-2409	2299	-236	-2381
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
17(S)	-1350	-2877	588	1045	-3189	-496	-920	-2963	-628	-2901	-2011	1860
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-2336	-8139	-325	-894	-1115	-701	-1378	*	*			
18(G)	-454	-832	-968	-1110	-2112	3143	-1211	-2091	-1317	-2264	-1691	-978
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-3098	-179	*	*			
19(H)	-898	-1313	-545	-482	-320	-1336	4297	-1552	-160	-1493	-1035	-579
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-3098	-179	*	*			
20(D)	-872	-1812	3234	432	-2215	-967	-433	-2172	-569	-2269	-1704	99
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-3098	-179	*	*			
21(E)	-766	-1695	521	2831	-2050	-1029	-293	-1804	-118	-1919	-1331	69
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-3098	-179	*	*			
22(Y)	-1337	-1229	-1681	-1596	1268	-1957	121	-918	-1294	-769	-585	-1229
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-109	-3775	*	*			
23(I)	-2294	-1931	-4749	-4227	-1724	-4227	-3320	2306	-3952	1990	-634	-3878
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
24(I)	-2801	-2299	-5406	-5003	-2108	-5164	-4649	3051	-4886	1593	-869	-4829
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
25(K)	-234	-2632	306	-500	-3007	-2141	-719	-2712	2540	-2619	-1730	-778
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
26(G)	-2184	-3900	796	392	-4174	2903	-1580	-4030	-1636	-3937	-3173	-967
—	-149	-501	233	42	-375	399	104	-625	210	-463	-722	276
—	-155	-3318	-9181	-3674	-118	-701	-1378	*	*			
27(K)	-3243	-3775	-4129	-2558	-4750	-3647	-1490	-4021	3681	-3617	-2982	-2368
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
28(K)	-1684	-2925	-1665	-979	-3407	-2535	-923	-3021	2737	-2865	-2032	202
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
29(V)	-2623	-2122	-5300	-4990	-2769	-5101	-5131	2388	-4945	-1532	-1474	-4790
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
30(A)	3309	-1828	-4057	-4294	-4382	656	-3657	-4147	-4169	-4428	-3497	-2821
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
31(V)	-2625	-2122	-5304	-4993	-2772	-5111	-5142	2881	-4950	-1532	-1474	-4796
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

32(I)	-2790	-2287	-5403	-5009	-2155	-5170	-4698	3324	-4899	1175	-912	-4835
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
33(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
34(Y)	-4838	-3766	-5229	-5579	1502	-5108	-1300	-3726	-5134	-3040	-3131	-3723
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
35(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
36(S)	-1473	-2007	-3647	-3780	-3430	-2363	-3314	228	-3616	-3373	-2876	-2840
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
37(Q)	-4589	-4392	-3927	-4146	-5099	-4221	-4099	-5973	-3840	-5564	-5304	-4230
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
38(G)	677	-2128	-3838	-4171	-4647	3536	-3816	-4506	-4340	-4749	-3857	-3009
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
39(H)	-2667	-3375	-2682	-2114	-3744	-3201	4738	-3782	-445	-3553	-2886	-2112
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
40(A)	3631	-2768	-4492	-4815	-4888	-2992	-4271	-4781	-4818	-5025	-4365	-3727
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
41(H)	-3103	-3404	-2950	-2573	-783	-3679	4549	-3407	-1372	-3071	-2715	-2454
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
42(A)	3357	-1795	-4134	-4277	-4057	-2118	-3548	-3549	-4035	-4024	-3192	-2817
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
43(Q)	-1061	1950	-2044	-1475	-1236	-2372	-1154	-789	-1218	1062	1123	743
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
44(N)	-4000	-4117	-3389	-3749	-5073	-3911	-4123	-6022	-4503	-5797	-5419	4397
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
45(L)	-4414	-3800	-5638	-5628	-2290	-4980	-4628	-1886	-5423	3316	-1236	-5514
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
46(R)	-1731	-3015	275	-931	-3487	-2518	-973	-3116	2321	-2955	-2123	224
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
47(D)	-2896	-4843	3855	944	-5037	-2600	-2082	-5082	-2528	-4903	-4373	-1209
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
48(S)	-1536	-2212	-2363	-2679	-4293	-2279	-3082	-4365	-3331	-4524	-3676	288
—	-148	-500	232	44	-381	398	105	-627	211	-465	-721	275
—	-155	-3318	-9181	-2405	-302	-701	-1378	*	*			
49(G)	-2521	-3968	1232	-911	-4849	3373	-2126	-4854	-2535	-4752	-4136	-53
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
50(V)	-2767	-2324	-5232	-4770	396	-4827	-3784	-36	-4546	848	-611	-4472
—	-148	-500	233	43	-381	399	106	-626	211	-466	-720	275
—	-148	-3381	-9181	-203	-2928	-701	-1378	*	*			
51(D)	-1684	-3285	2735	2014	-3554	-2196	-1177	-3350	92	-3279	-2427	692
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
52(V)	-3122	-2888	-5092	-5160	-3522	-4180	-4687	-905	-5060	-2626	-2570	-4662
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
53(V)	369	366	-3075	-2462	-883	-2557	-1420	1415	378	-757	-117	-2098
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
54(V)	-2624	-2122	-5302	-4991	-2772	-5108	-5139	2623	-4948	-1533	-1475	-4794
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
55(G)	929	-2107	-3852	-4182	-4633	3492	-3809	-4486	-4335	-4732	-3835	-2997
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
56(L)	-3427	-2938	-5791	-5325	-1449	-5374	-4410	-543	-5063	3041	-255	-5207
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

57(R)	-3040	-3724	-3266	82	-4620	-3470	-1396	-3905	804	-3529	-2874	-2133
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
58(K)	31	-2412	-803	1532	-2743	-1920	-559	-2483	1772	-2421	-1503	-556
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
59(G)	-2671	-4661	1614	587	-4832	3103	-1901	-4803	-2269	-4648	-4047	421
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
60(S)	-1499	-2308	-1932	-1859	-4006	1604	-2121	-3754	1362	-3793	-2945	-1833
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-247	-8139	-2699	-894	-1115	-701	-1378	*	*			
61(K)	1362	-2232	-619	-98	-2567	-427	-435	-2309	1599	-2265	-1349	1101
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-9	-7900	-8943	-894	-1115	-344	-2238	*	*			
62(S)	-1288	-1904	-3742	-4011	-4384	-2155	-3593	-4209	-3996	-4479	-3573	-2789
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
63(W)	726	-873	-3261	-2634	1926	-2567	-1425	660	-2252	-701	-68	-2174
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
64(E)	1527	-2404	212	1636	-2722	-1878	-556	-2474	1241	-2419	-1497	350
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
65(K)	-8	-2242	-895	770	-2502	-1963	-609	-2192	2589	22	-1353	-631
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
66(A)	3631	-2768	-4492	-4815	-4888	-2992	-4271	-4781	-4818	-5025	-4365	-3727
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
67(Q)	-1006	-2441	-869	1767	-2780	-1965	-586	-2510	1702	-2445	-1534	-603
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
68(A)	1489	-2393	167	1234	-2711	-1873	-547	-2462	895	-2408	-1485	1161
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
69(D)	2104	-2898	2124	985	-3163	-2096	1397	-2935	-693	-2897	-2025	-723
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
70(G)	-2294	-2898	-2521	-2885	-4852	3641	-3456	-5042	-3796	-5094	-4356	365
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
71(F)	-2596	-2266	-4685	-4188	3199	-4136	1018	505	-3812	1986	-405	-3595
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
72(K)	47	-2348	338	950	-2668	-1854	-512	-507	1721	-2364	-1438	-490
—	-149	-500	232	46	-381	399	105	-627	210	-466	-721	277
—	-155	-3318	-9181	-2159	-366	-701	-1378	*	*			
73(V)	-1810	-1639	-4149	-3689	-1869	-3417	-2822	29	-3369	320	-897	-3230
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
74(K)	847	-1093	-2131	-1554	304	127	-1127	-637	1445	645	1186	-1534
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
75(T)	-1284	-2794	1526	1290	-3096	-2041	1289	-2863	-548	-2808	-1914	-668
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
76(V)	-1089	-957	-3143	-2535	-943	-2618	-1496	1052	-2198	-792	1859	-146
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
77(W)	1606	-2321	-752	612	-2628	-323	-527	-2366	1480	-2331	-1416	-510
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
78(E)	-1509	-3540	1372	3127	-3861	-120	-1391	-3685	-1319	-3605	-2787	-900
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
79(A)	3390	-1868	-4092	-4341	-4332	-2153	-3680	-3942	-4157	-4333	-3471	-2869
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
80(V)	2003	-1721	-4449	-3995	-2160	-3763	-3240	1342	-3745	-1435	-1124	-3561
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
81(K)	1714	-2501	-959	446	-2858	-2043	-654	-2574	1964	-2506	-1609	-689
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

82(W)	265	-2347	815	432	-2663	634	-519	-2410	619	-2361	-1438	-495
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
83(A)	3391	-1860	-3998	-4279	-4411	-2128	-3684	-4207	-4197	-4490	-3565	-2837
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
84(D)	-2747	-4795	3813	396	-4912	-2496	-1935	-4905	-2324	-4735	-4166	-1079
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
85(V)	-2717	-2220	-5338	-4951	-2254	-5099	-4670	1963	-4844	1553	-1011	-4759
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
86(V)	-2635	-2129	-5306	-4970	-2652	-5125	-5011	2554	-4915	-354	-1368	-4781
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
87(M)	-1340	-1208	-3317	-2708	-968	-2860	-1708	577	-2346	932	4131	-2382
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
88(I)	-2566	-2177	-5017	-4470	669	-4496	-3487	2791	-4191	1116	1394	-4156
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
89(L)	-4414	-3800	-5638	-5628	-2290	-4980	-4628	-1886	-5423	3316	-1236	-5514
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
90(I)	1212	-1286	-3846	-3262	-1360	-3195	-2166	1616	-2918	1031	-493	-2824
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
91(P)	-1614	-2214	-3396	-3710	-4516	-2407	-3618	-4516	-3976	-4705	-3849	-2890
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
92(D)	-4580	-4701	4174	-3014	-5700	-3967	-3905	-6376	-4478	-6024	-5744	-3355
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
93(E)	-1123	-2199	-983	2715	-2589	-2046	-942	-2250	-625	-2356	1979	-870
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
94(H)	399	-1137	-2012	-14	1582	-2306	1600	246	-1252	190	-325	-1456
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
95(Q)	-2742	-3142	-2766	-2681	-2790	-3344	-2460	-160	-1802	-2456	-2353	-2682
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
96(A)	1981	-2315	-809	-268	-2645	-531	-579	-2374	232	-2350	-1445	-567
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
97(D)	491	-2351	1394	1381	-2671	-1854	1062	-2421	1010	-2367	-1440	-489
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
98(V)	-2039	-1706	-4456	-3939	-1846	-3939	-3049	1986	-3656	1460	-826	804
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
99(Y)	-4840	-3766	-5230	-5581	1898	-5109	-1300	-3727	-5135	-3041	-3132	-3723
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
100(E)	-163	-2353	-734	1681	-2674	-1859	888	-2422	1668	-792	-1443	777
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-257	-8139	-2649	-894	-1115	-701	-1378	*	*			
101(E)	1017	-2763	862	2042	-3060	-1913	-775	-2836	-495	-2773	-1886	1956
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-9	-7891	-8933	-894	-1115	-338	-2261	*	*			
102(E)	-944	-2422	863	2138	-2740	-436	-567	-2493	894	-2437	-1515	-518
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
103(I)	-2660	-2156	-5316	-4965	-2520	-5119	-4900	3165	-4894	297	-1251	-4775
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
104(E)	1068	-2341	-760	2003	628	-1887	876	-2380	1240	-2347	-1436	-529
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
105(P)	-343	-3144	1561	442	-3538	-489	-1216	-3329	-1038	-3274	-2420	-848
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
106(N)	-1173	-2375	-814	827	-2376	-2071	1767	-2279	-479	-2336	-1509	3151
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

107(M)	-3415	-2890	-5826	-5252	-1352	-5488	-4282	1361	-5022	2621	2728	-5181
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
108(K)	-1941	-3098	-1997	-1232	-3650	-2740	-1025	-3210	3059	-3010	-2208	499
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
109(P)	1129	-2426	-740	964	-2747	-1913	-589	-2491	1139	-2440	-1525	-552
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
110(G)	-2276	-2907	-2347	-2709	-4832	3554	-3349	-5005	-3678	-5053	-4315	1193
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
111(A)	1730	-2349	958	-198	-2661	-1868	-535	-2405	927	-2362	-1444	414
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
112(T)	1350	-1149	-14	-1461	-1155	-2314	-1111	758	-1275	-1024	1167	-1475
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
113(L)	-3333	-2796	-5806	-5293	-1506	-5535	-4502	1096	-5103	2935	-282	-5232
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
114(A)	1769	-1525	158	-857	-1603	148	-891	-1181	-752	187	-712	-1040
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
115(F)	-4110	-3437	-5436	-5431	4216	-5143	-2159	-1742	-5074	563	-1124	-4290
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
116(A)	3091	-1829	-3998	-4219	-4413	119	-3637	-4216	-4134	-4469	-3523	-2798
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
117(H)	-5197	-4539	-4720	-5009	-4036	-4506	5435	-6314	-4911	-5786	-5667	-4954
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
118(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
119(F)	-4044	-3387	-5534	-5444	4093	-5246	-2370	-1514	-5107	1089	-868	-4443
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
120(N)	885	-1899	-2020	-1781	-2956	-2135	-1925	-2602	-1809	3	-2052	3468
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
121(I)	-2673	-2169	-5324	-4969	-2477	-5123	-4876	3293	-4893	358	-1211	-4780
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
122(H)	-3381	-3705	-3197	-3491	-4166	638	5216	-5496	-3798	-5304	-4811	-3481
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
123(Y)	-4816	-3757	-5210	-5549	3410	-5097	2153	-3719	-5105	-3041	-3127	-3715
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
124(G)	-1065	-2519	948	-272	-2820	1844	998	-2566	972	-284	-1622	1553
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
125(Q)	-412	-2285	-2466	-2186	-2068	-2877	-2019	-1589	-1588	1526	-1121	-2187
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
126(I)	-2254	-1916	-4813	-4439	-2466	-4221	-3932	3248	-4248	-1515	-1324	-4044
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
127(K)	-888	-2234	334	1172	-2504	-1881	-546	-93	1370	-300	-1337	465
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
128(P)	715	-1925	-3618	-3897	-4464	653	-3594	-4274	-4053	-4520	-3596	-2770
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
129(P)	479	-2398	-1173	-637	-2915	-2106	-848	-2610	-289	-2586	-1713	-884
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
130(A)	1787	-2663	1377	529	-2976	-1992	-762	-2736	1785	-2680	-1776	-623
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-2336	-8139	-325	-894	-1115	-701	-1378	*	*			
131(F)	-1308	-1104	-2227	-2120	3516	-2093	-244	-196	-1891	64	66	-1626
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-3098	-179	*	*			

TABLE 9-continued

132(P)	-603	-937	-997	-1058	-1832	-1041	-1092	-1737	-1074	-1874	-1416	-992
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-3098	-179	*	*			
133(K)	-804	-1483	-564	-230	-1920	-1335	-101	-1605	2889	-1630	-1021	-349
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-109	-3775	*	*			
134(D)	-2405	-4159	3349	-651	-4260	-261	-1744	-4307	-1947	-4207	-3514	2151
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
135(I)	-2047	-1713	-4504	-3983	-1821	-3943	-3061	2461	-3697	1581	-797	-3593
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
136(D)	-2024	-3444	3495	-680	-3868	-2331	-1596	-44	-1632	-3675	-2915	685
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
137(V)	-3122	-2888	-5092	-5160	-3522	-4180	-4687	-905	-5060	-2626	-2570	-4662
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
138(I)	-53	-875	-3230	-2609	1867	-393	-1422	2613	-2236	-723	-81	-2157
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
139(M)	315	-2345	-4754	-4279	-1396	-4001	-3301	-697	-3877	816	4676	-3879
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
140(V)	-2623	-2122	-5301	-4990	-2770	-5102	-5132	2415	-4945	-1532	-1474	-4791
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
141(A)	3405	2528	-4529	-4796	-4340	-2257	-3851	-3901	-4447	-4351	-3532	-3057
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
142(P)	-4853	-4392	-5213	-5573	-5853	-4408	-5077	-6679	-5780	-6281	-6067	-5357
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
143(K)	-4484	-4357	-4380	-3992	-5413	-4236	-3307	-5555	3994	-5171	-4707	-3921
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
144(G)	2167	-1833	-3963	-4199	-4430	2715	-3642	-4236	-4146	-4489	-3540	-2795
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
145(P)	-2604	-2948	-4094	-4235	-3544	-3269	-3767	-3353	-3912	-3066	2095	-3659
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
146(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
147(H)	-2569	-3440	-1867	-1702	-3820	-2996	4731	-3830	634	-3639	-2963	-1838
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
148(T)	194	-1498	-3255	-2899	-2240	-2226	-2291	-1754	-2652	1634	-1430	-2330
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
149(V)	-3122	-2888	-5092	-5160	-3522	-4180	-4687	-905	-5060	-2626	-2570	-4662
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
150(R)	-4845	-4446	-5107	-4682	-5507	-4412	-3791	-5946	-2789	-5502	-5118	-4521
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
151(R)	-962	-2395	-777	1012	-2721	76	1031	-2459	-142	-2413	-1501	-560
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
152(E)	-902	-2032	-899	2078	-2228	-1934	-611	-1897	-259	-221	-1156	520
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
153(Y)	-4820	-3765	-5219	-5565	3303	-5093	-1317	-3703	-5127	-3017	-3111	-3732
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
154(V)	129	-1901	-989	821	-2060	-1969	-654	-1704	498	-52	-1037	-703
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
155(Q)	576	-2355	344	1156	-2675	-1856	-515	-508	1502	-2370	-1444	571
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
156(G)	-3239	-3889	516	-2361	-5355	3646	-3337	-5629	-3818	-5498	-4951	-2619
—	-149	-500	232	44	-381	399	105	-627	211	-466	-721	277
—	-155	-3318	-9181	-2159	-366	-701	-1378	*	*			

TABLE 9-continued

157(G)	753	-2516	-789	488	-2848	2300	-672	-2582	596	-2529	-1627	481
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
158(G)	-52	-2212	-3792	-4133	-4698	3627	-3843	-4580	-4356	-4812	-3937	-3058
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
159(V)	-2485	-2030	-5123	-4769	-2667	-4797	-4593	2349	-4661	-1545	-1424	-4502
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
160(P)	-2541	-3139	-2413	-2753	-4726	-2991	-3342	-5055	-3527	-5058	-4393	1199
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
161(C)	1577	3078	1357	-656	-2664	-219	891	-2359	-617	-2434	-1576	-891
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
162(L)	-2140	-2404	-3995	-3997	-2053	-3121	-3283	-1687	-3689	3041	-1200	-3360
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
163(I)	-2527	-2092	-5072	-4613	2047	-4674	-3911	2668	-4413	117	-841	-4323
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
164(A)	3631	-2768	-4492	-4815	-4888	-2992	-4271	-4781	-4818	-5025	-4365	-3727
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
165(V)	-2623	-2122	-5301	-4990	-2770	-5102	-5132	2426	-4946	-1532	-1474	-4791
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
166(H)	-495	-2631	903	-2051	722	-3242	3753	-2386	-2056	-2342	-1863	-2047
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
167(Q)	-4589	-4392	-3927	-4146	-5099	-4221	-4099	-5973	-3840	-5564	-5304	-4230
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
168(D)	-2873	-4605	3943	-902	-4948	-2633	-2157	-5087	-2604	-4922	-4387	428
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
169(A)	1776	-1612	-1274	138	-1698	-2092	-816	-1295	150	-1526	-780	-943
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
170(S)	-1545	-2420	1001	-1518	-4049	-2206	-2206	-3839	-2264	-3938	-3103	-1627
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
171(G)	-2999	-3461	-2978	-3207	-5161	3669	-3454	-5283	15	-5188	-4565	-3174
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
172(N)	-887	-2349	-736	911	-2668	-1860	-518	-2415	711	-2363	-1440	1880
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
173(A)	3631	-2768	-4492	-4815	-4888	-2992	-4271	-4781	-4818	-5025	-4365	-3727
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
174(K)	-1368	-2597	-1358	-720	-2953	-2298	1933	-2603	1975	851	-1706	-958
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
175(D)	-27	-2613	2320	1049	-2923	-1962	1973	-2684	544	-2624	-1712	1408
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
176(V)	-1096	-938	-3279	-2658	-899	-2643	-1513	1265	1006	1388	-124	-2232
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
177(A)	3342	-1826	-4064	-4295	-4368	111	-3651	-4129	-4159	-4412	-3484	-2820
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
178(L)	-4414	-3800	-5638	-5628	-2290	-4980	-4628	-1886	-5423	3316	-1236	-5514
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
179(S)	2216	-1831	-3961	-4157	-4409	656	-3608	-4213	-4076	-4462	-3514	-2782
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
180(Y)	-3634	-3050	-4918	-4872	36	-4597	-1405	223	-4437	-250	-1998	-3545
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
181(A)	3391	-1860	-3998	-4279	-4411	-2128	-3684	-4207	-4197	-4490	-3565	-2837
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			



TABLE 9-continued

182(K)	370	307	113	-351	-2280	-1925	-615	324	1888	-2040	-1194	-632
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
183(G)	2572	-2028	-3934	-4246	-4575	2752	-3783	-4406	-4316	-4661	-3751	-2958
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
184(I)	-2178	-1808	-4630	-4153	-2094	-4190	-3417	3121	-3909	311	-1023	698
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
185(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
186(G)	1392	2751	-4353	-4536	-4308	2864	-3681	-4084	-4233	-4354	-3425	-2859
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
187(G)	855	-1822	-3738	-3769	-4188	2507	-3358	-3950	-3667	-4196	-3283	-2667
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
188(R)	-3706	-3692	-4490	-3846	1391	-4057	-2273	-3795	-1906	-3355	-3181	-3458
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-155	-8139	-3345	-894	-1115	-701	-1378	*	*			
189(A)	2844	-1670	-3814	-3873	-4048	-1958	-3316	-3787	-3686	-4061	-3156	-2598
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-7992	-9034	-894	-1115	-1303	-750	*	*			
190(G)	-4176	-3995	-4855	-5222	-5686	3828	-4823	-6386	-5533	-6087	-5741	-4896
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-7992	-9034	-894	-1115	-422	-1980	*	*			
191(V)	-2496	-2036	-5139	-4788	-2680	-4825	-4637	2522	-4686	-1549	-1431	-4527
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
192(I)	-2760	-2307	-5270	-4785	1172	-4884	-3992	3346	-4576	662	-572	-4539
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
193(E)	454	-3086	-801	3279	-4125	-2346	-1868	-3919	-1868	-3932	-3156	-1291
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
194(T)	-2738	-3109	-4509	-4810	-4918	-3305	-4346	-4865	-4769	-5072	-4551	-3987
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
195(T)	-1323	-1975	-2766	-2978	-4207	-2152	-3105	-4004	-3295	-4229	-3354	134
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
196(F)	-4110	-3437	-5436	-5431	4216	-5143	-2159	-1742	-5074	563	-1124	-4290
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
197(K)	-111	-2844	-1470	1220	-3294	-2415	-860	-2939	2448	-2798	-1945	-1056
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
198(E)	545	-3735	1715	2880	-3981	-2308	-1442	-3818	-1408	-3725	-2924	-909
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
199(E)	-4574	-4665	-2714	3919	-5655	-3995	-3886	-6219	-4238	-5898	-5604	-3415
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
200(T)	-1211	1331	-3446	-2962	-1399	-2495	-1930	-869	-2610	-1374	-769	-2403
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
201(E)	-1941	-3222	-921	3293	-3618	-2473	-1316	-3186	916	-3225	-2465	-1147
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
202(T)	-1286	-1898	-3764	-4016	-4329	-2157	-3572	-4120	-3959	-4408	-3517	-2789
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
203(D)	-4580	-4701	4174	-3014	-5700	-3967	-3905	-6376	-4478	-6024	-5744	-3355
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
204(L)	-3705	-3122	-6060	-5527	-1359	-5814	-4569	1065	-5292	3069	-146	-5564
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
205(F)	-3777	-3220	-5271	-5259	4268	-4892	-2120	417	-4916	-1143	-1314	-4142
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
206(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

207(E)	-4574	-4665	-2714	3919	-5655	-3995	-3886	-6219	-4238	-5898	-5604	-3415
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
208(Q)	-3157	-3746	-3170	-2450	-4497	-3515	-1763	-4161	-443	-3809	-3189	-2392
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
209(A)	2672	-1334	-3318	-2853	-1740	371	-2072	483	-2577	-1549	-928	-2359
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
210(V)	-2620	-2125	-5293	-4983	-2756	-5076	-5100	1877	-4932	-1522	-1466	-4777
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
211(L)	-4414	-3800	-5638	-5628	-2290	-4980	-4628	-1886	-5423	3316	-1236	-5514
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
212(C)	-2243	5044	-4840	-4445	-1998	-3905	-3598	-31	-4138	449	-930	-3902
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
213(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
214(G)	677	-2128	-3838	-4171	-4647	-3536	-3816	-4506	-4340	-4749	-3857	-3009
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
215(V)	378	724	-3707	-3104	-1180	-2986	-1919	1210	-2734	1302	-359	-2627
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
216(M)	-948	-1407	-1515	156	-1452	-2164	1677	-1030	-821	-1302	1976	-1113
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
217(E)	1397	-2528	-725	2286	-2932	240	-791	-2681	328	-2645	-1744	-674
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
218(L)	-3705	-3122	-6060	-5527	-1359	-5814	-4569	1065	-5292	3069	-146	-5564
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
219(V)	-2600	-2108	-5251	-4894	-2568	-5025	-4783	2479	-4810	-1354	1358	-4683
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
220(K)	-1633	-2905	-1573	706	-3375	-2487	-900	-3003	2925	-2849	-2008	-1128
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
221(A)	2352	2066	-2593	-2000	-947	-2434	-1271	-486	-32	-832	714	-1817
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
222(G)	224	-1905	-3562	-3696	-3684	3361	-3297	-3220	-3625	81	-2886	-2733
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
223(F)	-4781	-3756	-5207	-5542	4341	-5070	-1342	-3653	-5111	-2971	-3065	-3743
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
224(E)	-2413	-4114	221	3465	-4392	-2485	-1689	-4248	-1608	-4112	-3396	-1094
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
225(T)	-1461	-1864	-3139	-2645	-2659	-2483	-2136	-1734	-1646	-2359	-1761	-2298
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
226(L)	-3831	-3266	-5314	-5148	-673	-5068	-2476	-1443	-4706	3059	-789	-4359
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
227(V)	-1819	-1960	-4426	-4359	-2977	-3037	-3800	-439	-4098	-2145	-1909	-3451
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
228(E)	-2863	-4790	1397	3563	-4990	-2594	-2061	-5021	-2476	-4848	-4298	-1204
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
229(A)	2686	-1916	-1440	275	-2529	-292	-1240	-2176	-998	-2345	1183	-1184
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
230(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
231(Y)	-4099	-3483	-4921	-5048	-109	-4705	-1565	-2914	-4494	-2334	2010	-3723
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

232(Q)	1711	-2410	-772	934	-2739	-1925	-604	-2477	-171	-2433	-1524	-574
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
233(P)	-3403	-4071	-1922	817	-5220	-3359	-3173	-5423	-3337	-5281	-4771	-2564
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
234(E)	-2870	-4786	1265	3587	-4993	-2600	-2068	-5026	-2483	-4852	-4303	-1212
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
235(M)	-3089	-2618	-5526	-4976	-1443	-5128	-4045	653	-4735	1429	4269	-4803
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
236(A)	3631	-2768	-4492	-4815	-4888	-2992	-4271	-4781	-4818	-5025	-4365	-3727
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
237(Y)	-4797	-3764	-5203	-5543	1114	-5069	-1339	-3694	-5111	-3013	-3107	-3741
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
238(F)	-3828	-3605	-4146	-4086	4292	-4207	-2060	-3492	774	-3071	-3005	-3556
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
239(E)	-2775	-4471	-511	3582	-4815	-2610	-2057	-4863	-2317	-4711	-4124	1234
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
240(C)	-1407	5023	-4397	-4323	-3016	-2468	-3398	-1251	-3952	-2540	-2082	-3044
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
241(L)	-3370	-2847	-5795	-5233	-1386	-5465	-4298	708	-5010	2859	1349	-5155
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
242(H)	-2519	-4224	-445	946	-4287	-2505	4583	-4377	-1764	-4237	-3571	2007
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
243(E)	-3177	2571	-2701	3711	-4851	-3438	-3479	-4765	-3558	-4932	-4406	-3081
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
244(L)	-85	-1333	-3893	-3280	-1111	-3185	-2083	1066	-2910	2310	1961	-2823
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
245(K)	-2513	-3173	-2941	-2370	-4402	-3094	-1824	-3895	3666	-3734	-3068	-2271
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-155	-3318	-9181	-196	-2974	-701	-1378	*	*			
246(L)	-3571	-3023	-5954	-5375	-1321	-5646	-4390	-632	-5138	2962	1671	-5358
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
247(I)	-2980	-2484	-5473	-5109	-1958	-5196	-4587	3728	-4915	267	-781	-4933
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
248(V)	-1685	-1668	-4095	-3732	-2081	-3082	-2893	-227	-3402	-1488	1383	-3123
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
249(D)	-2963	-4569	3864	-1039	-4953	-2751	-2187	-4998	767	-4822	-4260	-1424
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
250(L)	-2768	-2715	-4842	-4633	-1675	-3998	-3790	-1038	-4207	3056	-562	-4150
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
251(M)	-2822	-2356	-5342	-4861	-1759	-4985	-4151	2587	-4663	173	4005	-4649
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
252(Y)	-4562	-3630	-5142	-5401	1516	-4992	-1300	-3544	-4968	-2963	-2986	-3671
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
253(E)	-1959	-3457	-568	3135	-3841	-2347	-1410	-3622	-1165	-3547	-2751	-1000
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
254(G)	-347	-2818	-1215	201	-3253	2635	-921	-2921	1474	-2822	-1972	-1002
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
255(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
256(I)	-2042	-1769	-4321	-3740	-1316	-3753	-2668	3134	-3389	1017	1999	194
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

257(A)	1914	-1640	-1237	128	-1748	-661	-793	-1355	-577	-49	-817	-905
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
258(N)	365	-3809	1001	557	-4083	1196	-1518	-3930	-1535	-3838	-3055	3219
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
259(M)	-3656	-3159	-5816	-5350	-1349	-5421	-4248	-822	-4928	948	4920	-5248
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
260(R)	-1614	-1949	-2260	-1663	-886	-2765	-1089	-1596	-1089	360	-1133	2239
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
261(Y)	-1548	-2973	568	-509	-2846	-2207	1172	-2986	441	-2941	-2110	1645
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
262(S)	279	-1844	-3877	-4131	-4448	136	-3634	-4260	-4132	-4511	-3561	-2782
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
263(I)	-2653	-2149	-5311	-4961	-2538	-5114	-4905	2957	-4891	332	-1267	-4770
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
264(S)	-2212	-2711	-4019	-4348	-4697	-2899	-4045	-4988	-4527	-5102	-4364	-3492
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
265(N)	-2725	-4778	2906	990	-4896	-2486	-1922	-4885	-2320	-4718	-4140	3045
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
266(T)	-2061	-3396	-596	903	-4071	-2367	-1713	-3874	-1685	-3859	-3103	2125
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
267(A)	3410	-2035	-3979	-4290	-4573	659	-3798	-4400	-4332	-4660	-3754	-2978
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
268(E)	-2118	-3486	-1036	2964	-3935	-2588	-1284	-3596	1878	-3417	-2636	-1209
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
269(Y)	-4524	-3618	-5100	-5310	1910	-4972	-1299	-3522	634	-2951	-2965	-3649
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-155	-8139	-3345	-894	-1115	-701	-1378	*	*			
270(G)	-4176	-3995	-4855	-5222	-5686	3828	-4823	-6386	-5533	-6087	-5741	-4896
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-7992	-9034	-894	-1115	-422	-1980	*	*			
271(D)	-2710	-4705	3025	1828	-4880	1758	-1932	-4863	-2320	-4703	-4115	-1084
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
272(Y)	-2497	-2175	-4651	-4137	2447	-4046	-2215	255	-3766	892	1558	-3537
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
273(V)	-1425	-1250	-3480	-2894	-1283	-3035	-1955	691	-2570	-1	-429	-2568
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
274(T)	516	-1643	-1918	-1401	-2170	-2112	-1387	-1759	-1234	-2016	-1265	-1442
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
275(G)	677	-2128	-3838	-4171	-4647	3536	-3816	-4506	-4340	-4749	-3857	-3009
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
276(P)	-992	-2210	343	-359	-2447	-1960	-675	-2143	533	-2204	-1351	-651
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
277(R)	-1214	-2548	-1097	1072	175	-2145	-716	-2587	848	-2528	-1653	-795
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
278(V)	289	-2035	-5133	-4789	-2692	-4777	-4639	2142	-4689	-1561	-1443	-4511
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
279(I)	-2265	-1919	-4828	-4452	-2473	-4254	-3954	3155	-4265	-1516	-1326	-4066
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
280(D)	-1731	-3162	2329	-550	-3318	-2239	-1273	-3221	-1145	-3214	-2403	2295
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
281(E)	1097	-2699	1227	2368	-2994	-2011	-796	-2753	381	-2704	-1808	-640
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

282(E)	-166	-2372	859	1835	-2692	-1861	1182	-2444	490	-2388	-1462	590
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
283(T)	228	-1688	-3655	-3444	-3179	-2145	-2891	-2611	-3215	-3076	-2328	-2557
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
284(K)	-2991	-3623	-3848	-2331	-4472	-3512	-1356	-3763	2942	-3413	1860	-2188
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
285(E)	443	-2370	732	1690	-2691	-1863	-526	-2442	1639	-2385	-1460	-498
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
286(A)	1871	-2286	-843	814	-2570	-1928	-578	269	1096	-2279	-1391	-586
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
287(M)	-3656	-3159	-5816	-5350	-1349	-5421	-4248	-822	-4928	948	4920	-5248
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
288(K)	-1646	-2891	-1591	287	-3346	-526	-912	-2971	2831	-2832	-1997	-1146
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
289(E)	-172	-2394	367	2205	-2713	-487	-545	-2465	-134	-2409	-1485	1305
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
290(C)	1574	3024	-4584	-4122	-2155	-3932	-3330	1746	-3870	-1406	-1109	-3691
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
291(L)	-187	-2175	-4307	-3889	-898	-3779	-2344	-944	-3485	2855	-476	-3390
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
292(K)	862	-2347	143	1211	-2665	-1855	873	-2414	1692	-2362	917	-492
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
293(D)	-2148	-3878	2790	1765	-4119	-2356	-1511	-3962	-1467	-3852	-3075	24
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
294(I)	-2630	-2131	-5302	-4991	-2737	-5092	-5106	3464	-4941	-1495	-1447	-4789
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
295(Q)	346	-3134	-1818	-1401	-3862	-2760	-1314	-3433	1329	-3271	-2513	-1545
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
296(S)	-1354	-2895	1712	354	-3192	-2068	-914	-2967	-621	-2903	-2012	1817
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
297(G)	-4435	-4203	-5092	-5462	-5893	3834	-5028	-6627	-5765	-6297	-5970	-5141
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
298(E)	-437	-2374	-769	2013	-2697	-1895	-552	-2438	623	-2389	-1472	-536
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
299(F)	-4347	-3577	-4619	543	3858	-4820	-1320	-3438	-4609	-2900	-2894	-3532
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
300(A)	2827	-1603	-4068	-3628	-2047	-3165	-2823	1205	-3349	-1486	-1089	-3103
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
301(K)	-2364	-3363	-2464	163	-4038	-3041	-1188	-3501	2928	-3234	-2487	-1693
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
302(M)	-893	-2361	740	1780	-2680	-537	-524	-2429	930	-2376	1895	-498
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
303(W)	-2965	-2553	-4795	-4482	3045	-4315	-1779	-1426	-4093	92	-987	-3564
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
304(I)	-2621	-2136	-5246	-4859	-2364	-4987	-4577	3052	-4747	-250	1043	-4641
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
305(L)	684	-1319	-1741	761	-1375	-2223	-1037	-927	-1042	1693	-533	-1287
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
306(E)	364	-4165	621	3314	-4393	-2398	-1686	-4282	-1836	-4169	-3446	728
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			

TABLE 9-continued

307(N)	-939	979	-1235	-681	-1738	1352	935	-1357	-549	-1572	-816	2186
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
308(Q)	667	-2393	-773	511	-2721	-316	-544	-2465	1211	-2405	-1484	584
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
309(A)	2050	-1857	-1081	-526	-2013	-2012	188	-1645	-385	-1213	-1011	486
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
310(G)	-1848	-2469	-2089	-2292	1262	2944	-2358	-3563	-2904	-3628	-3017	2347
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
311(Y)	475	1019	-1606	-1042	225	-2192	-935	-946	-891	-1226	1222	357
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
312(P)	-87	-2372	-1362	756	-3738	-2205	-2007	-3445	-1924	-3575	-2761	-1555
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-2336	-8139	-325	-894	-1115	-701	-1378	*	*			
313(K)	-804	-1483	-564	-230	-1920	-1335	-101	-1605	2889	-1630	-1021	-349
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-3098	-179	*	*			
314(E)	-766	-1695	521	2831	-2050	-1029	-293	-1804	-118	-1919	-1331	69
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-109	-3775	*	*			
315(T)	-942	-2382	-739	1086	-2714	151	-581	-2459	-171	-2415	-1499	414
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
316(M)	-2196	-1920	-4499	-3891	1726	-3822	-2504	-645	-3523	1973	3030	-3442
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
317(H)	-883	-2314	-747	517	-2618	-1863	1714	-647	1272	-2322	-1408	1011
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
318(A)	2474	-2397	-816	-367	-2797	-273	-722	-2529	555	-2507	-1610	592
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
319(M)	-154	-986	-2485	-337	1024	-375	-1232	325	-444	867	1235	-1752
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
320(R)	-1311	-2432	-1349	-724	-2724	-2272	-799	-2361	613	-644	1079	976
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
321(R)	897	-2364	-833	905	-2678	-1930	-568	-2405	1293	-2366	1485	-575
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
322(N)	505	-2300	-750	523	-2598	121	-525	-594	485	95	-1395	1720
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
323(E)	444	-2266	-766	1488	-2551	-1871	-533	-2276	889	-2266	1474	1478
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
324(N)	1511	-1770	-728	-204	-2244	-1781	-426	282	601	-2121	-1133	1769
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
325(N)	1053	-3109	1756	1735	-3404	-2143	-1074	-3191	-846	-3124	-2254	2158
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
326(H)	-2064	-3071	-1245	-1267	-3262	-2570	4711	-3611	-1060	-3528	-2812	-1479
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
327(Q)	-891	-2294	332	948	-2585	-739	-537	-2316	-131	-138	-1391	-518
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
328(I)	-1632	-1661	-2846	-86	-1626	-2983	-1663	3240	-1327	-191	-722	-2160
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			
329(E)	-2734	-3605	-1382	3593	-3624	-2986	-2317	-3317	-2175	-3167	1983	-1898
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-2336	-8139	-325	-894	-1115	-701	-1378	*	*			
330(W)	-1530	-1265	-2068	-1964	479	-1810	-483	-1181	-1470	-968	-802	-1648
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-38	-5840	-6882	-894	-1115	-109	-3775	*	*			
331(K)	8	-2067	-905	437	-2275	-1941	-611	-307	2031	-2031	-1189	-636
—	-149	-500	233	43	-381	399	106	-626	210	-466	-720	275
—	-8	-8139	-9181	-894	-1115	-701	-1378	*	*			



TABLE 9-continued

2(M)	-4790	-4977	-4823	-4692	-4459	-3629	-4103	-4017	7200%
—	396	44	95	361	121	-368	-296	-251	
3(F)	-2278	-1503	-1798	-1617	-1350	-389	305	1335	8600%
—	394	45	96	359	117	-369	-294	-249	
4(A)	-1658	154	-383	-488	640	-3	-2038	-1421	8700%
—	394	45	96	359	117	-369	-294	-249	
5(C)	-1705	-451	-883	-631	-338	-50	-774	-133	8800%
—	394	45	96	359	118	-369	-295	-249	
6(S)	-1964	-1013	-1358	1715	-476	1117	-1320	-938	9000%
—	394	45	96	359	117	-369	-294	-249	
7(K)	-2010	1146	458	829	224	-2040	-2577	-1913	9100%
—	394	45	96	359	117	-369	-294	-249	
8(V)	-4600	-4417	-4628	-4080	-82	3023	-3952	-3510	9200%
—	394	45	96	359	117	-369	-294	-249	
9(Y)	-4920	-3835	-4458	-4313	-4533	-3643	-581	4349	9300%
—	394	45	96	359	117	-369	-294	-249	
10(Y)	-3206	-1513	-1078	-2258	1039	-2435	-2009	4185	9400%
—	394	45	96	359	117	-369	-294	-249	
11(D)	-2961	-1429	-2799	-2158	-2558	-3974	-4550	-3541	9500%
—	394	45	96	359	117	-369	-294	-249	
12(K)	-1960	-68	904	-67	-837	-1993	-2554	-1871	9600%
—	394	45	96	359	117	-369	-294	-249	
13(D)	-3046	-1551	-2987	-2292	-2742	-4201	-4759	-3709	9700%
—	394	45	96	359	117	-369	-294	-249	
14(C)	-3509	-3212	-3411	-2499	-1792	1507	-2796	-2431	9800%
—	394	45	96	359	117	-369	-294	-249	
15(D)	-2294	-489	-1186	53	1116	-2518	-3086	-2349	9900%
—	394	45	96	359	117	-369	-294	-249	
16(L)	-2862	-2089	-2316	-232	-1213	1306	-1645	-1304	10000%
—	394	45	96	359	117	-369	-294	-249	
17(S)	-2289	-489	-1184	2139	190	-2503	-3077	-2343	10100%
—	394	45	96	359	117	-369	-294	-249	
18(G)	-1499	-1202	-1421	-646	-774	-1550	-1916	-1919	10200%
—	394	45	96	359	117	-369	-294	-249	
19(H)	-1675	-363	-322	-934	-951	-1354	-725	107	10300%
—	394	45	96	359	117	-369	-294	-249	
20(D)	-1453	-184	-1141	-728	-973	-1814	-2146	-1646	10400%
—	394	45	96	359	117	-369	-294	-249	
21(E)	-1441	-4	-527	-653	-814	-1512	-1988	-1505	10500%
—	394	45	96	359	117	-369	-294	-249	
22(Y)	-2163	-1111	-1301	-1443	-1359	-932	592	3932	10600%
—	394	45	96	359	117	-369	-294	-249	
23(I)	94	-3538	-3812	-3411	-2247	1576	-2891	-2629	10700%
—	394	45	96	359	117	-369	-294	-249	
24(I)	-4788	-4454	-4829	-4493	-2764	1435	-3781	-3585	10800%
—	394	45	96	359	117	-369	-294	-249	
25(K)	-2231	2257	968	-1109	-1152	-2288	-2738	-2136	10900%
—	394	45	96	359	117	-369	-294	-249	
26(G)	-2810	-1	-2362	1069	-2220	-3530	-4130	-3229	11000%
—	396	44	96	358	116	-371	-296	-251	



TABLE 9-continued

27(K)	-3580	-1076	1318	-3119	-2876	-3817	-3395	-3374	12600%
—	394	45	96	359	117	-369	-294	-249	
28(K)	-2582	1301	804	-1564	1681	-2645	-2905	-2448	12700%
—	394	45	96	359	117	-369	-294	-249	
29(V)	-4868	-4890	-5101	-4482	-2619	3219	-4505	-3990	12800%
—	394	45	96	359	117	-369	-294	-249	
30(A)	-2904	-3694	-3937	-1470	59	-2957	-4610	-4522	12900%
—	394	45	96	359	117	-369	-294	-249	
31(V)	-4873	-4896	-5108	-4492	-2621	2896	-4512	-3997	13000%
—	394	45	96	359	117	-369	-294	-249	
32(I)	-4802	-4495	-4860	-4506	-2757	1192	-3838	-3622	13100%
—	394	45	96	359	117	-369	-294	-249	
33(G)	-4804	-5546	-5385	-4727	-4815	-5862	-4924	-5849	13200%
—	394	45	96	359	117	-369	-294	-249	
34(Y)	-4963	-3861	-4500	-4356	-4689	-3881	2986	4507	13300%
—	394	45	96	359	117	-369	-294	-249	
35(G)	-4804	-5546	-5385	-4727	-4815	-5862	-4924	-5849	13400%
—	394	45	96	359	117	-369	-294	-249	
36(S)	-3093	-3395	-3541	3475	-1885	-2307	-3927	-3474	13500%
—	394	45	96	359	117	-369	-294	-249	
37(Q)	-4693	4575	-3826	-4704	-4772	-5612	-4577	-4751	13600%
—	394	45	96	359	117	-369	-294	-249	
38(G)	-3149	-3871	-4137	-1784	-2005	-3297	-4725	-4735	13700%
—	394	45	96	359	117	-369	-294	-249	
39(H)	866	-1265	1506	-2614	-2557	-3469	-3282	-2908	13800%
—	394	45	96	359	117	-369	-294	-249	
40(A)	-3728	-4477	-4545	-2567	-2762	-3852	-4724	-4942	13900%
—	394	45	96	359	117	-369	-294	-249	
41(H)	-3764	2546	-1428	-2990	-2976	-3308	2269	-295	14000%
—	394	45	96	359	117	-369	-294	-249	
42(A)	-2900	-3608	-3823	217	-1660	-276	-4363	-4211	14100%
—	394	45	96	359	117	-369	-294	-249	
43(Q)	-2446	2895	-1441	-1392	-1005	-693	-1678	-1278	14200%
—	394	45	96	359	117	-369	-294	-249	
44(N)	-4479	-4255	-4592	-4115	-4312	-5371	-4650	-4731	14300%
—	394	45	96	359	117	-369	-294	-249	
45(L)	-4997	-4750	-5002	-5379	-4399	-2629	-3665	-3690	14400%
—	394	45	96	359	117	-369	-294	-249	
46(R)	-2603	256	2808	-1596	-1613	-2730	-2995	-2515	14500%
—	394	45	96	359	117	-369	-294	-249	
47(D)	-3196	-1786	-3536	-2501	-3007	-4517	-5004	-3956	14600%
—	394	45	96	359	117	-369	-294	-249	
48(S)	-3026	-2967	-3497	3508	-1962	-3259	-4477	-4066	14700%
—	393	45	95	360	118	-370	-295	-250	
49(G)	-3115	-1836	-3440	-2284	-2716	-4157	-4880	-3914	15400%
—	394	45	96	359	117	-369	-294	-249	
50(V)	-4518	-3980	-4367	-4081	-2716	3323	-3037	-2660	15500%
—	394	45	96	359	117	-369	-294	-249	
51(D)	-2505	-770	-1595	-1483	-1666	332	-3460	-2676	15700%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

52(V)	-4579	-4940	-4923	-4013	-3297	3796	-4414	-4190	15800%
—	394	45	96	359	117	-369	-294	-249	
53(V)	-2610	-1809	-2037	-1630	1166	2145	-1385	-343	15900%
—	394	45	96	359	117	-369	-294	-249	
54(V)	-4871	-4894	-5106	-4488	-2620	3088	-4511	-3996	16000%
—	394	45	96	359	117	-369	-294	-249	
55(G)	-3132	-3863	-4127	-1761	-1982	-3275	-4720	-4725	16100%
—	394	45	96	359	117	-369	-294	-249	
56(L)	-4820	-4126	-4691	-4757	-3351	883	-3184	-3234	16200%
—	394	45	96	359	117	-369	-294	-249	
57(R)	-3439	-978	3800	-2894	-2709	-3682	-3353	-3267	16300%
—	394	45	96	359	117	-369	-294	-249	
58(K)	1229	727	1079	-566	-893	-2041	-2579	-1915	16400%
—	394	45	96	359	117	-369	-294	-249	
59(G)	-3049	-1587	-3230	-2297	-2766	-4245	-4850	-3752	16500%
—	394	45	96	359	117	-369	-294	-249	
60(S)	-2827	-1794	-1902	2738	-1771	-2970	-3910	-3479	16600%
—	394	45	96	359	117	-369	-294	-249	
61(K)	-1861	886	-512	833	-740	-1868	-2441	-1767	16700%
—	394	45	96	359	117	-369	-294	-249	
62(S)	-2948	-3606	-3832	3517	228	-3028	-4600	-4451	16800%
—	394	45	96	359	117	-369	-294	-249	
63(W)	-2617	-1898	18	-1648	-972	983	4091	-958	16900%
—	394	45	96	359	117	-369	-294	-249	
64(E)	-1985	-100	-659	96	70	-2025	-2589	-1903	17000%
—	394	45	96	359	117	-369	-294	-249	
65(K)	-2052	692	-617	-889	-906	-361	-2455	-1836	17100%
—	394	45	96	359	117	-369	-294	-249	
66(A)	-3728	-4477	-4545	-2567	-2762	-3852	-4724	-4942	17200%
—	394	45	96	359	117	-369	-294	-249	
67(Q)	-2052	1923	873	-888	236	-630	-2596	-1949	17300%
—	394	45	96	359	117	-369	-294	-249	
68(A)	-1977	-90	-648	666	141	-2014	-2577	-1892	17400%
—	394	45	96	359	117	-369	-294	-249	
69(D)	-2329	-543	-1250	-1245	-1368	-2501	-3087	-530	17500%
—	394	45	96	359	117	-369	-294	-249	
70(G)	-3545	-3376	-4005	-2451	-2700	-3996	-4706	-4575	17600%
—	394	45	96	359	117	-369	-294	-249	
71(F)	-3961	-3157	-3524	-3277	-2509	-1337	-1621	-840	17700%
—	394	45	96	359	117	-369	-294	-249	
72(K)	-1947	672	436	806	687	-1970	-2533	-1851	17800%
—	393	45	95	359	119	-370	-295	-250	
73(V)	112	-3099	-3291	-2619	-767	3269	-2708	-2354	18400%
—	394	45	96	359	117	-369	-294	-249	
74(K)	-2401	-1174	-1547	-764	-172	-528	-1519	1413	18500%
—	394	45	96	359	117	-369	-294	-249	
75(T)	-2242	-427	-1095	1451	1827	-2411	-2986	-2264	18600%
—	394	45	96	359	117	-369	-294	-249	
76(V)	686	-1884	-2111	-1695	945	2346	-1458	-1106	18700%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

77(W)	-789	-73	421	23	-829	-1936	2212	-1843	18800%
—	394	45	96	359	117	-369	-294	-249	
78(E)	-2659	-1005	-1976	-400	-655	-3194	-3790	-2957	18900%
—	394	45	96	359	117	-369	-294	-249	
79(A)	-2948	-3730	-3919	-1525	931	-2894	-4580	-4483	19000%
—	394	45	96	359	117	-369	-294	-249	
80(V)	-3855	-3494	-3700	-2979	-58	2574	-3091	-2698	19100%
—	394	45	96	359	117	-369	-294	-249	
81(K)	-2135	-203	1088	428	-1032	-2148	-2652	-2027	19200%
—	394	45	96	359	117	-369	-294	-249	
82(W)	-1952	1955	-609	-382	147	-1966	2858	-1853	19300%
—	394	45	96	359	117	-369	-294	-249	
83(A)	-2929	-3729	-3959	706	-1718	-3001	-4636	-4534	19400%
—	394	45	96	359	117	-369	-294	-249	
84(D)	-3082	603	-3296	-2353	-2844	-4347	-4929	-3809	19500%
—	394	45	96	359	117	-369	-294	-249	
85(V)	-4771	-4509	-4836	-4427	-2688	2741	-3899	-3628	19600%
—	394	45	96	359	117	-369	-294	-249	
86(V)	-4852	-4798	-5038	-4487	-2622	3019	-4355	-3902	19700%
—	394	45	96	359	117	-369	-294	-249	
87(M)	-2878	250	-2265	-228	-1278	-506	-1629	-1313	19800%
—	394	45	96	359	117	-369	-294	-249	
88(I)	-4228	-3615	-3972	-3687	-2499	1692	-2860	-2711	19900%
—	394	45	96	359	117	-369	-294	-249	
89(L)	-4997	-4750	-5002	-5379	-4399	-2629	-3665	-3690	20000%
—	394	45	96	359	117	-369	-294	-249	
90(I)	-3211	-2583	-2782	-2308	1598	1299	-2020	-1688	20100%
—	394	45	96	359	117	-369	-294	-249	
91(P)	3993	-3625	-3900	666	-2068	-3354	-4610	-4474	20200%
—	394	45	96	359	117	-369	-294	-249	
92(D)	-4501	-3870	-4926	-4440	-4750	-5894	-4922	-5231	20300%
—	394	45	96	359	117	-369	-294	-249	
93(E)	-2250	-554	-1093	463	932	-1902	-2660	-2064	20400%
—	394	45	96	359	117	-369	-294	-249	
94(H)	-2374	1474	-1479	-94	-905	896	-1557	-1158	20500%
—	394	45	96	359	117	-369	-294	-249	
95(Q)	-3710	4317	-1866	-2894	-2844	-2559	-3295	-2711	20600%
—	394	45	96	359	117	-369	-294	-249	
96(A)	1217	711	445	447	-874	-1951	-2540	-1883	20700%
—	394	45	96	359	117	-369	-294	-249	
97(D)	-1947	1017	362	-760	250	-623	-2535	-1852	20800%
—	394	45	96	359	117	-369	-294	-249	
98(V)	-3870	-3351	-3565	-3105	-2000	2330	-2796	-2442	20900%
—	394	45	96	359	117	-369	-294	-249	
99(Y)	-4964	-3861	-4501	-4357	-4690	-3883	3325	4377	21000%
—	394	45	96	359	117	-369	-294	-249	
100(E)	-1952	890	286	-766	238	-1975	-2536	-1856	21100%
—	394	45	96	359	117	-369	-294	-249	
101(E)	-2143	-136	-1056	265	-1185	-2377	-2948	-2207	21200%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

102(E)	-1994	1767	-673	109	-885	-1023	-2605	-1917	21300%
—	394	45	96	359	117	-369	-294	-249	
103(I)	-4828	-4705	-4975	-4470	-2642	2240	-4202	-3814	21400%
—	394	45	96	359	117	-369	-294	-249	
104(E)	-1983	881	-618	-804	-855	-1954	-2530	-1862	21500%
—	394	45	96	359	117	-369	-294	-249	
105(P)	2974	-812	-1635	469	-1644	-2849	-3462	-2693	21600%
—	394	45	96	359	117	-369	-294	-249	
106(N)	-2218	-415	-957	-1093	-1120	-198	-2486	647	21700%
—	394	45	96	359	117	-369	-294	-249	
107(M)	-4778	-4005	-4613	-4776	-3292	69	-3071	-3194	21800%
—	394	45	96	359	117	-369	-294	-249	
108(K)	-2766	1457	1261	-1817	-90	-2858	-3002	-2622	21900%
—	394	45	96	359	117	-369	-294	-249	
109(P)	1941	1446	-655	-480	-913	-2050	-2610	-1935	22000%
—	394	45	96	359	117	-369	-294	-249	
110(G)	-3507	-3243	-3937	-2418	-2674	-3974	-4703	-4521	22100%
—	394	45	96	359	117	-369	-294	-249	
111(A)	-1966	788	-630	790	-840	-303	-2540	-1863	22200%
—	394	45	96	359	117	-369	-294	-249	
112(T)	-2388	-1111	-1501	334	1843	354	-1581	-1182	22300%
—	394	45	96	359	117	-369	-294	-249	
113(L)	-4857	-4172	-4762	-4864	-3236	506	-3264	-3351	22400%
—	394	45	96	359	117	-369	-294	-249	
114(A)	-2228	660	-1135	-1111	-913	1305	-1913	-1442	22500%
—	394	45	96	359	117	-369	-294	-249	
115(F)	-4871	-3987	-4561	-4547	-4016	-2374	-1356	-292	22600%
—	394	45	96	359	117	-369	-294	-249	
116(A)	-2896	-3656	-3927	1514	-1679	-2983	-4632	-4539	22700%
—	394	45	96	359	117	-369	-294	-249	
117(H)	-4960	-5011	-4732	-5391	-5395	-6022	-4063	-3641	22800%
—	394	45	96	359	117	-369	-294	-249	
118(G)	-4804	-5546	-5385	-4727	-4815	-5862	-4924	-5849	22900%
—	394	45	96	359	117	-369	-294	-249	
119(F)	-4880	-3998	-4592	-4639	-3934	-2200	-1536	-523	23000%
—	394	45	96	359	117	-369	-294	-249	
120(N)	-2633	-1676	-2141	413	-1437	-2139	-3194	-2737	23100%
—	394	45	96	359	117	-369	-294	-249	
121(I)	-4824	-4681	-4961	-4472	-2653	1969	-4158	-3791	23200%
—	394	45	96	359	117	-369	-294	-249	
122(H)	-4185	-3770	-3879	-3508	-3702	-4793	-4170	-3759	23300%
—	394	45	96	359	117	-369	-294	-249	
123(Y)	-4955	-3851	-4483	-4344	-4669	-3870	-547	3677	23400%
—	394	45	96	359	117	-369	-294	-249	
124(G)	-2090	-229	-802	-938	-1011	-2133	-2708	-2021	23500%
—	394	45	96	359	117	-369	-294	-249	
125(Q)	-3153	3585	-1718	-2137	-1964	-1719	-2789	-2414	23600%
—	394	45	96	359	117	-369	-294	-249	
126(I)	-4259	-4063	-4255	230	-2280	2003	-3673	-3237	23700%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

127(K)	-1974	655	-646	-794	-827	1255	-2448	-1798	23800%
—	394	45	96	359	117	-369	-294	-249	
128(P)	3775	-3593	-3911	-1550	-1770	-3067	-4647	-4548	23900%
—	394	45	96	359	117	-369	-294	-249	
129(P)	2238	1247	2195	51	-1147	-2184	-2757	-2174	24000%
—	394	45	96	359	117	-369	-294	-249	
130(A)	-2161	-319	-936	297	-1120	-2285	-2853	-2146	24100%
—	394	45	96	359	117	-369	-294	-249	
131(F)	-2278	-1503	-1798	-1617	-1350	-389	305	1335	24200%
—	394	45	96	359	117	-369	-294	-249	
132(P)	3539	-1065	-1192	-789	-866	-1383	-1765	-1661	24300%
—	394	45	96	359	117	-369	-294	-249	
133(K)	-1569	232	698	-786	-759	-1358	-1637	-1317	24400%
—	394	45	96	359	117	-369	-294	-249	
134(D)	-2936	-1416	-2754	-2102	-2471	-3802	-4324	637	24500%
—	394	45	96	359	117	-369	-294	-249	
135(I)	-3873	-3371	-3587	-342	-2009	1904	-2784	-2441	24600%
—	394	45	96	359	117	-369	-294	-249	
136(D)	-2782	-1248	-2305	478	-2088	-3196	-3911	-3098	24700%
—	394	45	96	359	117	-369	-294	-249	
137(V)	-4579	-4940	-4923	-4013	-3297	3796	-4414	-4190	24800%
—	394	45	96	359	117	-369	-294	-249	
138(I)	-2608	-1885	-2086	-1633	276	-271	-1325	844	24900%
—	394	45	96	359	117	-369	-294	-249	
139(M)	-3994	-3361	-3676	-3242	-2531	-1114	-2746	-2629	25000%
—	394	45	96	359	117	-369	-294	-249	
140(V)	-4869	-4890	-5102	-4483	-2619	3206	-4506	-3991	25100%
—	394	45	96	359	117	-369	-294	-249	
141(A)	-3052	-3976	-4112	-1643	-1844	-2929	-4572	-4519	25200%
—	394	45	96	359	117	-369	-294	-249	
142(P)	4310	-5648	-5396	-5166	-5194	-6092	-4900	-5786	25300%
—	394	45	96	359	117	-369	-294	-249	
143(K)	-4535	-3079	-2169	-4529	-4408	-5264	-4403	-4729	25400%
—	394	45	96	359	117	-369	-294	-249	
144(G)	-2898	-3661	-3939	910	-1682	-2994	-4647	-4556	25500%
—	394	45	96	359	117	-369	-294	-249	
145(P)	4036	-3912	-3822	-2883	-2963	-3249	-4027	-3787	25600%
—	394	45	96	359	117	-369	-294	-249	
146(G)	-4804	-5546	-5385	-4727	-4815	-5862	-4924	-5849	25700%
—	394	45	96	359	117	-369	-294	-249	
147(H)	1551	-1305	-748	-2470	-2510	-3482	-3434	-2990	25800%
—	394	45	96	359	117	-369	-294	-249	
148(T)	-2747	-2399	-2684	567	2687	-1484	-2682	-2351	25900%
—	394	45	96	359	117	-369	-294	-249	
149(V)	-4579	-4940	-4923	-4013	-3297	3796	-4414	-4190	26000%
—	394	45	96	359	117	-369	-294	-249	
150(R)	-4754	-3672	4219	-4989	-4832	-5644	-4538	-4993	26100%
—	394	45	96	359	117	-369	-294	-249	
151(R)	-2018	-128	2308	1224	-66	-2023	-2585	-1919	26200%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

152(E)	-2024	816	-736	-858	1303	-287	-2295	537	26300%
—	394	45	96	359	117	-369	-294	-249	
153(Y)	-4959	-3868	-4500	-4356	-4679	-3867	-565	4052	26400%
—	394	45	96	359	117	-369	-294	-249	
154(V)	-2057	695	-796	443	-344	1871	-2192	-1626	26500%
—	394	45	96	359	117	-369	-294	-249	
155(Q)	-1949	1878	419	-764	-822	-1976	-2538	-1856	26600%
—	394	45	96	359	117	-369	-294	-249	
156(G)	-3905	-3187	-4377	-3211	-3532	-4837	-4895	-4826	26700%
—	393	45	95	359	117	-368	-295	-250	
157(G)	-2112	-224	471	-962	-1024	-2149	-2694	-2033	27300%
—	394	45	96	359	117	-369	-294	-249	
158(G)	-3216	-3901	-4170	-1874	-2095	-3384	-4734	-4766	27400%
—	394	45	96	359	117	-369	-294	-249	
159(V)	-4648	-4554	-4752	-4115	825	2986	-4159	-3678	27500%
—	394	45	96	359	117	-369	-294	-249	
160(P)	4031	-3244	-3757	-2665	-2911	-4148	-4583	-4362	27600%
—	394	45	96	359	117	-369	-294	-249	
161(C)	-2199	-545	-1093	872	-1043	-1946	-2701	-2102	27700%
—	394	45	96	359	117	-369	-294	-249	
162(L)	-3626	-3433	-3567	480	-2414	-1973	-3145	-2755	27800%
—	394	45	96	359	117	-369	-294	-249	
163(I)	-4439	-4023	-4320	-3916	-2488	2176	-3342	-3040	27900%
—	394	45	96	359	117	-369	-294	-249	
164(A)	-3728	-4477	-4545	-2567	-2762	-3852	-4724	-4942	28000%
—	394	45	96	359	117	-369	-294	-249	
165(V)	-4869	-4891	-5102	-4483	-2619	3200	-4506	-3991	28100%
—	394	45	96	359	117	-369	-294	-249	
166(H)	-3330	-1815	-2362	-2318	-2233	-2272	-981	3315	28200%
—	394	45	96	359	117	-369	-294	-249	
167(Q)	-4693	4575	-3826	-4704	-4772	-5612	-4577	-4751	28300%
—	394	45	96	359	117	-369	-294	-249	
168(D)	-3235	-1872	-3575	-2522	-3009	-4491	-4932	-3946	28400%
—	394	45	96	359	117	-369	-294	-249	
169(A)	1212	-538	-1013	-1056	-894	1275	-1968	635	28500%
—	394	45	96	359	117	-369	-294	-249	
170(S)	-2814	-1909	-2758	2666	2313	-3045	-4143	-3560	28600%
—	394	45	96	359	117	-369	-294	-249	
171(G)	-3946	-3312	-3140	-3128	-3317	-4492	-4622	-4749	28700%
—	394	45	96	359	117	-369	-294	-249	
172(N)	-1954	436	799	671	1230	-539	-2534	-1855	28800%
—	394	45	96	359	117	-369	-294	-249	
173(A)	-3728	-4477	-4545	-2567	-2762	-3852	-4724	-4942	28900%
—	394	45	96	359	117	-369	-294	-249	
174(K)	-2360	859	1012	-1273	1094	-2245	-2690	-2165	29000%
—	394	45	96	359	117	-369	-294	-249	
175(D)	-2115	345	-869	-969	-1058	-2231	-2793	-2087	29100%
—	394	45	96	359	117	-369	-294	-249	
176(V)	-2688	-1950	-2151	-1725	-311	1669	-1425	827	29200%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

177(A)	-2903	-3689	-3929	-1469	409	-2949	-4599	-4508	29300%
—	394	45	96	359	117	-369	-294	-249	
178(L)	-4997	-4750	-5002	-5379	-4399	-2629	-3665	-3690	29400%
—	394	45	96	359	117	-369	-294	-249	
179(S)	-2892	-3613	-3895	2686	-1675	-2982	-4623	-4523	29500%
—	394	45	96	359	117	-369	-294	-249	
180(Y)	-4494	-3522	-4004	-3782	-3536	-2621	2928	4349	29600%
—	394	45	96	359	117	-369	-294	-249	
181(A)	-2929	-3729	-3959	706	-1718	-3001	-4636	-4534	29700%
—	394	45	96	359	117	-369	-294	-249	
182(K)	-2022	-205	-736	1541	14	-1628	-2330	-1726	29800%
—	394	45	96	359	117	-369	-294	-249	
183(G)	-3070	-3837	-4092	-1679	-1898	-3191	-4701	-4686	29900%
—	394	45	96	359	117	-369	-294	-249	
184(I)	-4099	-3656	-3864	-3388	-2148	1742	-3147	-2761	30000%
—	394	45	96	359	117	-369	-294	-249	
185(G)	-4804	-5546	-5385	-4727	-4815	-5862	-4924	-5849	30100%
—	394	45	96	359	117	-369	-294	-249	
186(G)	-2890	-3744	-3957	712	-1656	-2914	-4553	-4470	30200%
—	394	45	96	359	117	-369	-294	-249	
187(G)	986	-3302	-3621	-1441	2334	-2867	-4408	-4236	30300%
—	394	45	96	359	117	-369	-294	-249	
188(R)	-4263	-2675	3948	-3768	-3671	-3813	-2293	-1328	30400%
—	394	45	96	359	117	-369	-294	-249	
189(A)	-2734	-3301	-3572	1088	1907	-2713	-4286	-4136	30500%
—	394	45	96	359	117	-369	-294	-249	
190(G)	-4606	-5312	-5178	-4461	-4560	-5613	-4754	-5635	30600%
—	394	45	96	359	117	-369	-294	-249	
191(V)	-4668	-4584	-4783	-4148	629	2919	-4191	-3706	30700%
—	394	45	96	359	117	-369	-294	-249	
192(I)	-4535	-4008	-4400	-4134	-2703	757	-3223	-3041	30800%
—	394	45	96	359	117	-369	-294	-249	
193(E)	1208	-1533	-2450	-1842	-2097	-3305	-4121	-3395	30900%
—	394	45	96	359	117	-369	-294	-249	
194(T)	-3998	-4580	-4545	-2999	4033	-4113	-4684	-4915	31000%
—	394	45	96	359	117	-369	-294	-249	
195(T)	-2901	-2988	-3387	-113	3742	-2969	-4405	-4119	31100%
—	394	45	96	359	117	-369	-294	-249	
196(F)	-4871	-3987	-4561	-4547	-4016	-2374	-1356	-292	31200%
—	394	45	96	359	117	-369	-294	-249	
197(K)	-2475	767	2054	-1421	-1432	-2544	-2858	-2356	31300%
—	394	45	96	359	117	-369	-294	-249	
198(E)	-2713	1281	-2087	-1777	-2041	-3331	-3909	-3046	31400%
—	394	45	96	359	117	-369	-294	-249	
199(E)	-4513	-3838	-4570	-4456	-4726	-5786	-4878	-5197	31500%
—	394	45	96	359	117	-369	-294	-249	
200(T)	-2815	-2309	-2526	-1685	3305	195	-1933	1430	31600%
—	394	45	96	359	117	-369	-294	-249	
201(E)	-2749	-923	-1064	-1790	-1902	-171	-3398	-2802	31700%
—	394	45	96	359	117	-369	-294	-249	





TABLE 9-continued

227(V)	-3600	-3879	-4011	-2397	2510	2999	-3974	-3608	34300%
—	394	45	96	359	117	-369	-294	-249	
228(E)	-3182	-1761	-3454	-2476	-2968	-4462	-4951	-3920	34400%
—	394	45	96	359	117	-369	-294	-249	
229(A)	-2355	-905	-1425	512	-1158	-1817	-2697	-2174	34500%
—	394	45	96	359	117	-369	-294	-249	
230(G)	-4804	-5546	-5385	-4727	-4815	-5862	-4924	-5849	34600%
—	394	45	96	359	117	-369	-294	-249	
231(Y)	-4707	-3735	-4111	-4065	-4068	-3172	-847	4618	34700%
—	394	45	96	359	117	-369	-294	-249	
232(Q)	-2035	2086	345	902	-923	-2042	-2608	-1941	34800%
—	394	45	96	359	117	-369	-294	-249	
233(P)	4045	-2989	-3760	-3320	-3624	-4817	-4763	-4636	34900%
—	394	45	96	359	117	-369	-294	-249	
234(E)	-3188	-1768	-3458	-2484	-2974	-4467	-4950	-3925	35000%
—	394	45	96	359	117	-369	-294	-249	
235(M)	-4610	-3915	-4423	-4378	-2995	1140	-3054	-3074	35100%
—	394	45	96	359	117	-369	-294	-249	
236(A)	-3728	-4477	-4545	-2567	-2762	-3852	-4724	-4942	35200%
—	394	45	96	359	117	-369	-294	-249	
237(Y)	-4951	-3876	-4497	-4354	-4666	-3859	-588	4723	35300%
—	394	45	96	359	117	-369	-294	-249	
238(F)	-4434	-3287	-2952	-3868	-3858	-3571	-1593	-494	35400%
—	394	45	96	359	117	-369	-294	-249	
239(E)	-3182	-1755	-3103	-2442	-2884	-4306	-4753	-3820	35500%
—	394	45	96	359	117	-369	-294	-249	
240(C)	-3133	-3588	-3744	-1803	1473	1125	-3677	-3390	35600%
—	394	45	96	359	117	-369	-294	-249	
241(L)	-4777	-4026	-4622	-4755	-3254	814	-3103	-3213	35700%
—	394	45	96	359	117	-369	-294	-249	
242(H)	-3009	-1439	-2361	-2209	-2569	-3893	-4267	-3353	35800%
—	394	45	96	359	117	-369	-294	-249	
243(E)	-4005	-3370	-3802	-3269	-3451	-4260	-4554	-4524	35900%
—	394	45	96	359	117	-369	-294	-249	
244(L)	-3170	-2501	-2721	-2289	-113	436	-1859	-1558	36000%
—	394	45	96	359	117	-369	-294	-249	
245(K)	-3377	-1447	-616	-2537	751	-3485	-3625	-3471	36100%
—	394	45	96	359	117	-369	-294	-249	
246(L)	-4852	-4044	-4689	-4963	-3436	742	-3082	-3239	36300%
—	394	45	96	359	117	-369	-294	-249	
247(I)	-4833	-4427	-4799	-4598	-2949	-64	-3627	-3397	36400%
—	394	45	96	359	117	-369	-294	-249	
248(V)	-3419	-3145	-3320	367	-1807	3332	-2874	-2504	36500%
—	394	45	96	359	117	-369	-294	-249	
249(D)	-3314	-1891	-3072	-2624	-3060	-4467	-4770	-3962	36600%
—	394	45	96	359	117	-369	-294	-249	
250(L)	-4179	-3740	-3989	-3399	699	-1545	-3154	-3367	36700%
—	394	45	96	359	117	-369	-294	-249	
251(M)	-4601	-4076	-4487	-4251	-2764	766	-3321	-3210	36800%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

252(Y)	-4868	-3786	-4393	381	-4432	-3662	2413	4375	36900%
—	394	45	96	359	117	-369	-294	-249	
253(E)	-2716	1879	-1666	-1757	692	-3162	-3709	-2960	37000%
—	394	45	96	359	117	-369	-294	-249	
254(G)	-2459	-486	658	-1397	-1435	-2521	-2923	-2378	37100%
—	394	45	96	359	117	-369	-294	-249	
255(G)	-4804	-5546	-5385	-4727	-4815	-5862	-4924	-5849	37200%
—	394	45	96	359	117	-369	-294	-249	
256(I)	-3653	-2958	-3221	-2884	-1980	-344	-2325	-2073	37300%
—	394	45	96	359	117	-369	-294	-249	
257(A)	-2155	-498	-993	1037	162	-1149	-2002	624	37400%
—	394	45	96	359	117	-369	-294	-249	
258(N)	-2763	-1148	-2243	-1845	-2131	-3433	-4027	-3144	37500%
—	394	45	96	359	117	-369	-294	-249	
259(M)	-4838	-4039	-4539	-4860	-3558	-1557	-3044	-3030	37600%
—	394	45	96	359	117	-369	-294	-249	
260(R)	-2814	-1215	2408	-1789	-1535	-1468	1995	1546	37700%
—	394	45	96	359	117	-369	-294	-249	
261(Y)	-2446	-659	-1264	-1389	-1509	-2582	-2924	3695	37800%
—	394	45	96	359	117	-369	-294	-249	
262(S)	-2903	-3648	-3936	3391	-1692	-3009	-4661	-4566	37900%
—	394	45	96	359	117	-369	-294	-249	
263(I)	-4827	-4713	-4978	-4466	-2636	2521	-4218	-3821	38000%
—	394	45	96	359	117	-369	-294	-249	
264(S)	-3638	-4203	-4355	3681	-2664	-3902	-4616	-4605	38100%
—	394	45	96	359	117	-369	-294	-249	
265(N)	-3069	-1612	-3311	-2334	-2821	-4325	-4919	-3794	38200%
—	394	45	96	359	117	-369	-294	-249	
266(T)	-2850	-1369	-2277	-1897	3157	-3351	-4055	-3271	38300%
—	394	45	96	359	117	-369	-294	-249	
267(A)	-3080	-3858	-4099	-1690	-1908	-3194	-4697	-4687	38400%
—	394	45	96	359	117	-369	-294	-249	
268(E)	-2827	1323	-773	-1923	-2032	-3199	-3455	-2917	38500%
—	394	45	96	359	117	-369	-294	-249	
269(Y)	-4847	-3741	-4299	-4199	-4391	-3637	2997	4211	38600%
—	394	45	96	359	117	-369	-294	-249	
270(G)	-4606	-5312	-5178	-4461	-4560	-5613	-4754	-5635	38700%
—	394	45	96	359	117	-369	-294	-249	
271(D)	-3073	-1621	-3297	-2330	-2809	-4301	-4894	-3793	38800%
—	394	45	96	359	117	-369	-294	-249	
272(Y)	-3886	-3109	-3468	-3181	-2410	-1282	-1615	3508	38900%
—	394	45	96	359	117	-369	-294	-249	
273(V)	-3060	942	-2514	-2129	1701	2578	-1907	-1553	39000%
—	394	45	96	359	117	-369	-294	-249	
274(T)	-2421	-1149	887	1341	2345	822	-2454	-2006	39100%
—	394	45	96	359	117	-369	-294	-249	
275(G)	-3149	-3871	-4137	-1784	-2005	-3297	-4725	-4735	39200%
—	394	45	96	359	117	-369	-294	-249	
276(P)	2813	-260	-802	465	-939	-873	-2467	1093	39300%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

277(R)	-2228	-273	2862	417	-1133	-2191	-2671	-2084	39400%
—	394	45	96	359	117	-369	-294	-249	
278(V)	-4649	-4585	-4784	-4102	-2487	3125	-4202	-3717	39500%
—	394	45	96	359	117	-369	-294	-249	
279(I)	-4279	-4082	-4274	226	-2288	2182	-3688	-3250	39600%
—	394	45	96	359	117	-369	-294	-249	
280(D)	-2573	-899	-1742	-1561	1851	-2804	-3366	1327	39700%
—	394	45	96	359	117	-369	-294	-249	
281(E)	-2190	-358	-992	-1065	-1162	-2310	-2885	1152	39800%
—	394	45	96	359	117	-369	-294	-249	
282(E)	478	1356	-620	116	-837	-1994	-2555	-1871	39900%
—	394	45	96	359	117	-369	-294	-249	
283(T)	-2821	-2916	-3214	2251	2366	1397	-3549	-3270	40000%
—	394	45	96	359	117	-369	-294	-249	
284(K)	-3430	-937	2705	-2874	-2650	-3556	-3267	-3189	40100%
—	394	45	96	359	117	-369	-294	-249	
285(E)	-1960	1120	437	106	-836	-1992	-2552	-1869	40200%
—	394	45	96	359	117	-369	-294	-249	
286(A)	-2019	-136	1056	-62	-881	-1889	-2488	-1849	40300%
—	394	45	96	359	117	-369	-294	-249	
287(M)	-4838	-4039	-4539	-4860	-3558	-1557	-3044	-3030	40400%
—	394	45	96	359	117	-369	-294	-249	
288(K)	-2554	-472	1762	-1527	-1524	-2597	-2885	1245	40500%
—	394	45	96	359	117	-369	-294	-249	
289(E)	-1975	663	831	-795	72	-2015	-2577	-1891	40600%
—	394	45	96	359	117	-369	-294	-249	
290(C)	-3957	-3613	-3805	-3144	-2046	2342	-3118	-2720	40700%
—	394	45	96	359	117	-369	-294	-249	
291(L)	-3782	-3025	-3298	-2972	-2345	-1269	-1846	1565	40800%
—	394	45	96	359	117	-369	-294	-249	
292(K)	-1949	889	-603	-763	783	-1968	-2532	-1851	40900%
—	394	45	96	359	117	-369	-294	-249	
293(D)	-2782	-1139	2142	-1879	-2163	-3473	-4024	-3155	41000%
—	394	45	96	359	117	-369	-294	-249	
294(I)	-4862	-4869	-5086	-4473	-2627	2071	-4467	-3968	41100%
—	394	45	96	359	117	-369	-294	-249	
295(Q)	-2936	3817	-430	-2018	-2031	-3060	-3278	-2908	41200%
—	394	45	96	359	117	-369	-294	-249	
296(S)	-2288	724	-1177	1978	-96	-2508	-3076	-2340	41300%
—	394	45	96	359	117	-369	-294	-249	
297(G)	-4804	-5546	-5385	-4727	-4815	-5862	-4924	-5849	41400%
—	394	45	96	359	117	-369	-294	-249	
298(E)	-1991	-97	777	829	1488	-1999	-2559	-1890	41500%
—	394	45	96	359	117	-369	-294	-249	
299(F)	-4750	-3628	-4209	-4078	-4237	-3546	-601	2917	41600%
—	394	45	96	359	117	-369	-294	-249	
300(A)	-3432	-3073	-3298	-2387	197	1100	-2812	-2447	41700%
—	394	45	96	359	117	-369	-294	-249	
301(K)	-3047	-758	2488	-458	-2152	-3194	-3159	-2895	41800%
—	394	45	96	359	117	-369	-294	-249	

TABLE 9-continued

302(M)	-1958	-66	722	775	-833	-1982	-2545	-1864	41900%
—	394	45	96	359	117	-369	-294	-249	
303(W)	-4179	-3330	-3743	-3475	-2878	664	4754	-84	42000%
—	394	45	96	359	117	-369	-294	-249	
304(I)	-4713	-4488	-4769	-4301	-2597	2384	-3934	-3596	42100%
—	394	45	96	359	117	-369	-294	-249	
305(L)	-2325	-915	-1349	767	-152	84	-1756	-1329	42200%
—	394	45	96	359	117	-369	-294	-249	
306(E)	-2893	-1339	-2626	-2045	-2402	-3767	-4362	-3401	42300%
—	394	45	96	359	117	-369	-294	-249	
307(N)	-2155	-482	165	-1022	-880	30	-1990	1446	42400%
—	394	45	96	359	117	-369	-294	-249	
308(Q)	-1989	2135	1528	-812	-868	-2019	-2566	-1895	42500%
—	394	45	96	359	117	-369	-294	-249	
309(A)	-2103	-346	1657	-953	306	-261	-2169	-1624	42600%
—	394	45	96	359	117	-369	-294	-249	
310(G)	-3167	-2550	-3185	-1986	-2162	-3006	-2814	-1874	42700%
—	394	45	96	359	117	-369	-294	-249	
311(Y)	-2267	1002	1577	-1172	-888	-800	-1730	2446	42800%
—	394	45	96	359	117	-369	-294	-249	
312(P)	3598	-1697	-2362	-1566	286	-2803	-3832	-3277	42900%
—	394	45	96	359	117	-369	-294	-249	
313(K)	-1569	232	698	-786	-759	-1358	-1637	-1317	43000%
—	394	45	96	359	117	-369	-294	-249	
314(E)	-1441	-4	-527	-653	-814	-1512	-1988	-1505	43100%
—	394	45	96	359	117	-369	-294	-249	
315(T)	-2004	-128	839	1365	1730	-2017	-2592	-1915	43200%
—	394	45	96	359	117	-369	-294	-249	
316(M)	-3673	-2938	-3257	-2944	-2114	326	-2014	1662	43300%
—	394	45	96	359	117	-369	-294	-249	
317(H)	472	-69	433	-772	1411	-299	-2507	-1836	43400%
—	394	45	96	359	117	-369	-294	-249	
318(A)	-2110	837	-805	-138	-1006	-2092	-2699	-2039	43500%
—	394	45	96	359	117	-369	-294	-249	
319(M)	-2474	-1419	1020	-1455	670	-411	831	535	43600%
—	394	45	96	359	117	-369	-294	-249	
320(R)	597	-382	2908	-1246	-1219	-2044	-2579	-2061	43700%
—	394	45	96	359	117	-369	-294	-249	
321(R)	-2020	-177	2045	95	-893	-1984	-2543	-1892	43800%
—	394	45	96	359	117	-369	-294	-249	
322(N)	-1957	348	224	551	-821	-1910	-2497	-1828	43900%
—	394	45	96	359	117	-369	-294	-249	
323(E)	-1963	682	-629	-781	12	-279	-2472	476	44000%
—	394	45	96	359	117	-369	-294	-249	
324(N)	-1827	1398	447	-748	-766	-1713	-1779	-1595	44100%
—	394	45	96	359	117	-369	-294	-249	
325(N)	-2417	-657	-1430	-1361	197	-2727	-3303	-2540	44200%
—	394	45	96	359	117	-369	-294	-249	
326(H)	-2961	1288	-1287	670	-2133	-3161	-3310	-2601	44300%
—	394	45	96	359	117	-369	-294	-249	



TABLE 9-continued

352(D)	34	-182	-708	541	-74	-281	-2188	-1605	46900%
—	394	45	96	359	117	-369	-294	-249	
353(K)	-2548	-407	1469	-1628	-1517	-119	-2497	1619	47000%
—	394	45	96	359	117	-369	-294	-249	
354(N)	1327	-1460	-2119	1182	-1362	-2558	-3601	-3046	47100%
—	*	*	*	*	*	*	*	*	

HMMER2.0 [2.2 g]	File format version: a unique identifier for this save file format.
NAME Functionally Verified KARIs	Name of the profile HMM
LENG 354	Model length: the number of match states in the model.
ALPH Amino	Symbol alphabet: This determines the symbol alphabet and the size of the symbol emission probability distributions. I.Amino, the alphabet size is set to 20 and the symbol alphabet to "ACDEFGHIKLMNPQRSTVWY" (alphabetic order).
MAP yes	Map annotation flag: If set to yes, each line of data for the match state/consensus column in the main section of the file is followed by an extra number. This number gives the index of the alignment column that the match state was made from. This information provides a "map" of the match states (1 . . . M) onto the columns of the alignment (1.alen). It is used for quickly aligning the model back to the original alignment, e.g. when using hmmlalign-mapali.
COM hmmbuild-n Functionally Verified KARIs exp-KARI.hmm exp-KARI_mod.aln	Command line for every HMMER command that modifies the save file: This one means that hmmbuild (default parameters) was applied to generate the save file.
COM hmmlcalibrate exp-KARI.hmm	Command line for every HMMER command that modifies the save file: This one means that hmmlcalibrate (default parameters) was applied to the save profile.
NSEQ 25	Sequence number: the number of sequences the HMM was trained on
DATE Mon Dec 8 17:34:51 2008	Creation date: When was the save file generated.
XT -8455 -4 -1000 -1000	Eight "special" transitions for controlling parts of the algorithm-specific parts of the Plan7 model. The null probability used to convert these back to model probabilities is 1.0. The order of the eight fields is N->B, N->N, E->C, E->J, C->T, C->C, J->B, J->J.
-8455 -4 -8455 -4	The transition probability distribution for the null model (single G state).
NULL -4 -8455	The extreme value distribution parameters $\mu$ and $\lambda$ respectively; both floating point values. These values are set when the model is calibrated with hmmlcalibrate. They are used to determine E-values of bit scores.
NULE 595 -1558 85 338 -294 453	
-1158 197 249 902 -1085 -142	
-21 -313 45 531 201 384 -1998 -644	
EVD -333.712708 0.110102	

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 70

<210> SEQ ID NO 1  
 <211> LENGTH: 30  
 <212> TYPE: DNA  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: primer

<400> SEQUENCE: 1

tgatgaacat cttcgcgtat tcgcccgtcct

30

<210> SEQ ID NO 2  
 <211> LENGTH: 68  
 <212> TYPE: DNA  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: forward primer for library C  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (24)..(25)  
 <223> OTHER INFORMATION: n is a, c, g, or t  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (33)..(34)  
 <223> OTHER INFORMATION: n is a, c, g, or t

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<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (39)..(40)  
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 2

gcgtagacgt gactgttggc ctgnntaaag gcnnggctnn ctgggccaag gctgaagccc 60  
acggcttg 68

<210> SEQ ID NO 3  
<211> LENGTH: 68  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: forward primer for library E  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (24)..(25)  
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 3

gcgtagacgt gactgttggc ctgnntaaag gctcggttac cgttgccaag gctgaagccc 60  
acggcttg 68

<210> SEQ ID NO 4  
<211> LENGTH: 68  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: forward primer for library F  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (33)..(34)  
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 4

gcgtagacgt gactgttggc ctgcgtaaag gcnntgctac cgttgccaag gctgaagccc 60  
acggcttg 68

<210> SEQ ID NO 5  
<211> LENGTH: 68  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: forward primer for library G  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (39)..(40)  
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 5

gcgtagacgt gactgttggc ctgcgtaaag gctcggttnn tgttgccaag gctgaagccc 60  
acggcttg 68

<210> SEQ ID NO 6  
<211> LENGTH: 68  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: forward primer for library H  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (24)..(25)  
<223> OTHER INFORMATION: n is a, c, g, or t

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (33)..(34)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (39)..(40)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 6

gcgtagacgt gactgttgge ctgnntaaag gcnntgctnn tgttgccaag gctgaagccc 60
acggcttg 68

<210> SEQ ID NO 7
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: sequencing primer (forward)

<400> SEQUENCE: 7

aagattagcg gatcctacct 20

<210> SEQ ID NO 8
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: sequencing primer (reverse)

<400> SEQUENCE: 8

aacagccaag cttttagttc 20

<210> SEQ ID NO 9
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Methanococcus aeolicus

<400> SEQUENCE: 9

Met Lys Val Phe Tyr Asp Ser Asp Phe Lys Leu Asp Ala Leu Lys Glu
1 5 10 15

Lys Thr Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly Arg Ala Gln Ser
20 25 30

Leu Asn Met Lys Asp Ser Gly Leu Asn Val Val Val Gly Leu Arg Lys
35 40 45

Asn Gly Ala Ser Trp Glu Asn Ala Lys Ala Asp Gly His Asn Val Met
50 55 60

Thr Ile Glu Glu Ala Ala Glu Lys Ala Asp Ile Ile His Ile Leu Ile
65 70 75 80

Pro Asp Glu Leu Gln Ala Glu Val Tyr Glu Ser Gln Ile Lys Pro Tyr
85 90 95

Leu Lys Glu Gly Lys Thr Leu Ser Phe Ser His Gly Phe Asn Ile His
100 105 110

Tyr Gly Phe Ile Val Pro Pro Lys Gly Val Asn Val Val Leu Val Ala
115 120 125

Pro Lys Ser Pro Gly Lys Met Val Arg Arg Thr Tyr Glu Glu Gly Phe
130 135 140

Gly Val Pro Gly Leu Ile Cys Ile Glu Ile Asp Ala Thr Asn Asn Ala
145 150 155 160

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Phe Asp Ile Val Ser Ala Met Ala Lys Gly Ile Gly Leu Ser Arg Ala  
 165 170 175  
 Gly Val Ile Gln Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Val Thr Glu Leu Ile Lys Ala  
 195 200 205  
 Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Thr Cys His Glu Leu Lys Leu Ile Val Asp Leu Ile Tyr Gln  
 225 230 235 240  
 Lys Gly Phe Lys Asn Met Trp Asn Asp Val Ser Asn Thr Ala Glu Tyr  
 245 250 255  
 Gly Gly Leu Thr Arg Arg Ser Arg Ile Val Thr Ala Asp Ser Lys Ala  
 260 265 270  
 Ala Met Lys Glu Ile Leu Lys Glu Ile Gln Asp Gly Arg Phe Thr Lys  
 275 280 285  
 Glu Phe Val Leu Glu Lys Gln Val Asn His Ala His Leu Lys Ala Met  
 290 295 300  
 Arg Arg Ile Glu Gly Asp Leu Gln Ile Glu Glu Val Gly Ala Lys Leu  
 305 310 315 320  
 Arg Lys Met Cys Gly Leu Glu Lys Glu Glu  
 325 330

&lt;210&gt; SEQ ID NO 10

&lt;211&gt; LENGTH: 330

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Methanococcus maripaludis

&lt;400&gt; SEQUENCE: 10

Met Lys Val Phe Tyr Asp Ser Asp Phe Lys Leu Asp Ala Leu Lys Glu  
 1 5 10 15  
 Lys Thr Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly Arg Ala Gln Ser  
 20 25 30  
 Leu Asn Met Lys Asp Ser Gly Leu Asn Val Val Val Gly Leu Arg Lys  
 35 40 45  
 Asn Gly Ala Ser Trp Asn Asn Ala Lys Ala Asp Gly His Asn Val Met  
 50 55 60  
 Thr Ile Glu Glu Ala Ala Glu Lys Ala Asp Ile Ile His Ile Leu Ile  
 65 70 75 80  
 Pro Asp Glu Leu Gln Ala Glu Val Tyr Glu Ser Gln Ile Lys Pro Tyr  
 85 90 95  
 Leu Lys Glu Gly Lys Thr Leu Ser Phe Ser His Gly Phe Asn Ile His  
 100 105 110  
 Tyr Gly Phe Ile Val Pro Pro Lys Gly Val Asn Val Val Leu Val Ala  
 115 120 125  
 Pro Lys Ser Pro Gly Lys Met Val Arg Arg Thr Tyr Glu Glu Gly Phe  
 130 135 140  
 Gly Val Pro Gly Leu Ile Cys Ile Glu Ile Asp Ala Thr Asn Asn Ala  
 145 150 155 160  
 Phe Asp Ile Val Ser Ala Met Ala Lys Gly Ile Gly Leu Ser Arg Ala  
 165 170 175  
 Gly Val Ile Gln Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe

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180                                    185                                    190

Gly Glu Gln Ala Val Leu Cys Gly Gly Val Thr Glu Leu Ile Lys Ala  
195                                    200                                    205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
210                                    215                                    220

Phe Glu Thr Cys His Glu Leu Lys Leu Ile Val Asp Leu Ile Tyr Gln  
225                                    230                                    235                                    240

Lys Gly Phe Lys Asn Met Trp Asn Asp Val Ser Asn Thr Ala Glu Tyr  
245                                    250                                    255

Gly Gly Leu Thr Arg Arg Ser Arg Ile Val Thr Ala Asp Ser Lys Ala  
260                                    265                                    270

Ala Met Lys Glu Ile Leu Arg Glu Ile Gln Asp Gly Arg Phe Thr Lys  
275                                    280                                    285

Glu Phe Leu Leu Glu Lys Gln Val Ser Tyr Ala His Leu Lys Ser Met  
290                                    295                                    300

Arg Arg Leu Glu Gly Asp Leu Gln Ile Glu Glu Val Gly Ala Lys Leu  
305                                    310                                    315                                    320

Arg Lys Met Cys Gly Leu Glu Lys Glu Glu  
325                                    330

<210> SEQ ID NO 11  
 <211> LENGTH: 330  
 <212> TYPE: PRT  
 <213> ORGANISM: Methanococcus vannielii

<400> SEQUENCE: 11

Met Lys Val Phe Tyr Asp Ala Asp Ile Lys Leu Asp Ala Leu Lys Ser  
1                                    5                                    10                                    15

Lys Thr Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly Arg Ala Gln Ser  
20                                    25                                    30

Leu Asn Met Lys Asp Ser Gly Leu Asn Val Val Val Gly Leu Arg Lys  
35                                    40                                    45

Asn Gly Ala Ser Trp Glu Asn Ala Lys Asn Asp Gly His Glu Val Leu  
50                                    55                                    60

Thr Ile Glu Glu Ala Ser Lys Lys Ala Asp Ile Ile His Ile Leu Ile  
65                                    70                                    75                                    80

Pro Asp Glu Leu Gln Ala Glu Val Tyr Glu Ser Gln Ile Lys Pro Tyr  
85                                    90                                    95

Leu Thr Glu Gly Lys Thr Leu Ser Phe Ser His Gly Phe Asn Ile His  
100                                    105                                    110

Tyr Gly Phe Ile Ile Pro Pro Lys Gly Val Asn Val Val Leu Val Ala  
115                                    120                                    125

Pro Lys Ser Pro Gly Lys Met Val Arg Lys Thr Tyr Glu Glu Gly Phe  
130                                    135                                    140

Gly Val Pro Gly Leu Ile Cys Ile Glu Val Asp Ala Thr Asn Thr Ala  
145                                    150                                    155                                    160

Phe Glu Thr Val Ser Ala Met Ala Lys Gly Ile Gly Leu Ser Arg Ala  
165                                    170                                    175

Gly Val Ile Gln Thr Thr Phe Arg Glu Glu Thr Glu Thr Asp Leu Phe  
180                                    185                                    190

Gly Glu Gln Ala Val Leu Cys Gly Gly Val Thr Glu Leu Ile Lys Ala  
195                                    200                                    205

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Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ser Pro Glu Met Ala Tyr  
 210 215 220

Phe Glu Thr Cys His Glu Leu Lys Leu Ile Val Asp Leu Ile Tyr Gln  
 225 230 235 240

Lys Gly Phe Lys Asn Met Trp His Asp Val Ser Asn Thr Ala Glu Tyr  
 245 250 255

Gly Gly Leu Thr Arg Arg Ser Arg Ile Val Thr Ala Asp Ser Lys Ala  
 260 265 270

Ala Met Lys Glu Ile Leu Lys Glu Ile Gln Asp Gly Arg Phe Thr Lys  
 275 280 285

Glu Phe Val Leu Glu Asn Gln Ala Lys Met Ala His Leu Lys Ala Met  
 290 295 300

Arg Arg Leu Glu Gly Glu Leu Gln Ile Glu Glu Val Gly Ser Lys Leu  
 305 310 315 320

Arg Lys Met Cys Gly Leu Glu Lys Asp Glu  
 325 330

&lt;210&gt; SEQ ID NO 12

&lt;211&gt; LENGTH: 349

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Saccharomyces cerevisiae*

&lt;400&gt; SEQUENCE: 12

Met Leu Lys Gln Ile Asn Phe Gly Gly Thr Val Glu Thr Val Tyr Glu  
 1 5 10 15

Arg Ala Asp Trp Pro Arg Glu Lys Leu Leu Asp Tyr Phe Lys Asn Asp  
 20 25 30

Thr Phe Ala Leu Ile Gly Tyr Gly Ser Gln Gly Tyr Gly Gln Gly Leu  
 35 40 45

Asn Leu Arg Asp Asn Gly Leu Asn Val Ile Ile Gly Val Arg Lys Asp  
 50 55 60

Gly Ala Ser Trp Lys Ala Ala Ile Glu Asp Gly Trp Val Pro Gly Lys  
 65 70 75 80

Asn Leu Phe Thr Val Glu Asp Ala Ile Lys Arg Gly Ser Tyr Val Met  
 85 90 95

Asn Leu Leu Ser Asp Ala Ala Gln Ser Glu Thr Trp Pro Ala Ile Lys  
 100 105 110

Pro Leu Leu Thr Lys Gly Lys Thr Leu Tyr Phe Ser His Gly Phe Ser  
 115 120 125

Pro Val Phe Lys Asp Leu Thr His Val Glu Pro Pro Lys Asp Leu Asp  
 130 135 140

Val Ile Leu Val Ala Pro Lys Gly Ser Gly Arg Thr Val Arg Ser Leu  
 145 150 155 160

Phe Lys Glu Gly Arg Gly Ile Asn Ser Ser Tyr Ala Val Trp Asn Asp  
 165 170 175

Val Thr Gly Lys Ala His Glu Lys Ala Gln Ala Leu Ala Val Ala Ile  
 180 185 190

Gly Ser Gly Tyr Val Tyr Gln Thr Thr Phe Glu Arg Glu Val Asn Ser  
 195 200 205

Asp Leu Tyr Gly Glu Arg Gly Cys Leu Met Gly Gly Ile His Gly Met  
 210 215 220

Phe Leu Ala Gln Tyr Asp Val Leu Arg Glu Asn Gly His Ser Pro Ser  
 225 230 235 240

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Glu Ala Phe Asn Glu Thr Val Glu Glu Ala Thr Gln Ser Leu Tyr Pro  
 245 250 255  
 Leu Ile Gly Lys Tyr Gly Met Asp Tyr Met Tyr Asp Ala Cys Ser Thr  
 260 265 270  
 Thr Ala Arg Arg Gly Ala Leu Asp Trp Tyr Pro Ile Phe Lys Asn Ala  
 275 280 285  
 Leu Lys Pro Val Phe Gln Asp Leu Tyr Glu Ser Thr Lys Asn Gly Thr  
 290 295 300  
 Glu Thr Lys Arg Ser Leu Glu Phe Asn Ser Gln Pro Asp Tyr Arg Glu  
 305 310 315 320  
 Lys Leu Glu Lys Glu Leu Asp Thr Ile Arg Asn Met Glu Ile Trp Lys  
 325 330 335  
 Val Gly Lys Glu Val Arg Lys Leu Arg Pro Glu Asn Gln  
 340 345

<210> SEQ ID NO 13  
 <211> LENGTH: 335  
 <212> TYPE: PRT  
 <213> ORGANISM: Sulfolobus solfataricus

<400> SEQUENCE: 13

Met Lys Cys Thr Ser Lys Ile Tyr Thr Asp Asn Asp Ala Asn Leu Asp  
 1 5 10 15  
 Leu Ile Lys Gly Lys Arg Ile Ala Val Leu Gly Tyr Gly Ser Gln Gly  
 20 25 30  
 Arg Ala Trp Ala Gln Asn Leu Arg Asp Ser Gly Leu Asn Val Val Val  
 35 40 45  
 Gly Leu Glu Arg Glu Gly Lys Ser Trp Glu Leu Ala Lys Ser Asp Gly  
 50 55 60  
 Ile Thr Pro Leu His Thr Lys Asp Ala Val Lys Asp Ala Asp Ile Ile  
 65 70 75 80  
 Ile Phe Leu Val Pro Asp Met Val Gln Arg Thr Leu Trp Leu Glu Ser  
 85 90 95  
 Val Gln Pro Tyr Met Lys Lys Gly Ala Asp Leu Val Phe Ala His Gly  
 100 105 110  
 Phe Asn Ile His Tyr Lys Leu Ile Asp Pro Pro Lys Asp Ser Asp Val  
 115 120 125  
 Tyr Met Ile Ala Pro Lys Gly Pro Gly Pro Thr Val Arg Glu Tyr Tyr  
 130 135 140  
 Lys Ala Gly Gly Gly Val Pro Ala Leu Val Ala Val His Gln Asp Val  
 145 150 155 160  
 Ser Gly Thr Ala Leu His Lys Ala Leu Ala Ile Ala Lys Gly Ile Gly  
 165 170 175  
 Ala Thr Arg Ala Gly Val Ile Pro Thr Thr Phe Lys Glu Glu Thr Glu  
 180 185 190  
 Thr Asp Leu Phe Gly Glu Gln Val Ile Leu Val Gly Gly Ile Met Glu  
 195 200 205  
 Leu Met Arg Ala Ala Phe Glu Thr Leu Val Glu Glu Gly Tyr Gln Pro  
 210 215 220  
 Glu Val Ala Tyr Phe Glu Thr Ile Asn Glu Leu Lys Met Leu Val Asp  
 225 230 235 240  
 Leu Val Tyr Glu Lys Gly Ile Ser Gly Met Leu Lys Ala Val Ser Asp

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245                    250                    255  
 Thr Ala Lys Tyr Gly Gly Met Thr Val Gly Lys Phe Val Ile Asp Glu  
 260                    265                    270  
 Ser Val Arg Lys Arg Met Lys Glu Ala Leu Gln Arg Ile Lys Ser Gly  
 275                    280                    285  
 Lys Phe Ala Glu Glu Trp Val Glu Glu Tyr Gly Arg Gly Met Pro Thr  
 290                    295                    300  
 Val Val Asn Gly Leu Ser Asn Val Gln Asn Ser Leu Glu Glu Lys Ile  
 305                    310                    315                    320  
 Gly Asn Gln Leu Arg Asp Leu Val Gln Lys Gly Lys Pro Lys Ser  
 325                    330                    335  
  
 <210> SEQ ID NO 14  
 <211> LENGTH: 328  
 <212> TYPE: PRT  
 <213> ORGANISM: Pyrobaculum aerophilum  
  
 <400> SEQUENCE: 14  
 Met Ala Lys Ile Tyr Thr Asp Arg Glu Ala Ser Leu Glu Pro Leu Lys  
 1                    5                    10                    15  
 Gly Lys Thr Ile Ala Val Ile Gly Tyr Gly Ile Gln Gly Arg Ala Gln  
 20                    25                    30  
 Ala Leu Asn Leu Arg Asp Ser Gly Leu Glu Val Ile Ile Gly Leu Arg  
 35                    40                    45  
 Arg Gly Gly Lys Ser Trp Glu Leu Ala Thr Ser Glu Gly Phe Arg Val  
 50                    55                    60  
 Tyr Glu Ile Gly Glu Ala Val Arg Lys Ala Asp Val Ile Leu Val Leu  
 65                    70                    75                    80  
 Ile Pro Asp Met Glu Gln Pro Lys Val Trp Gln Glu Gln Ile Ala Pro  
 85                    90                    95  
 Asn Leu Lys Glu Gly Val Val Val Asp Phe Ala His Gly Phe Asn Val  
 100                    105                    110  
 His Phe Gly Leu Ile Lys Pro Pro Lys Asn Ile Asp Val Ile Met Val  
 115                    120                    125  
 Ala Pro Lys Ala Pro Gly Lys Ala Val Arg Glu Glu Tyr Leu Ala Gly  
 130                    135                    140  
 Arg Gly Val Pro Ala Leu Val Ala Val Tyr Gln Asp Tyr Ser Gly Ser  
 145                    150                    155                    160  
 Ala Leu Lys Tyr Ala Leu Ala Leu Ala Lys Gly Ile Gly Ala Thr Arg  
 165                    170                    175  
 Ala Gly Val Ile Glu Thr Thr Phe Ala Glu Glu Thr Glu Thr Asp Leu  
 180                    185                    190  
 Ile Gly Glu Gln Ile Val Leu Val Gly Gly Leu Met Glu Leu Ile Lys  
 195                    200                    205  
 Lys Gly Phe Glu Val Leu Val Glu Met Gly Tyr Gln Pro Glu Val Ala  
 210                    215                    220  
 Tyr Phe Glu Val Leu Asn Glu Ala Lys Leu Ile Met Asp Leu Ile Trp  
 225                    230                    235                    240  
 Gln Arg Gly Ile Tyr Gly Met Leu Asn Gly Val Ser Asp Thr Ala Lys  
 245                    250                    255  
 Tyr Gly Gly Leu Thr Val Gly Pro Arg Val Ile Asp Glu Asn Val Lys  
 260                    265                    270

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Arg Lys Met Lys Glu Ala Ala Met Arg Val Lys Ser Gly Glu Phe Ala  
275 280 285

Lys Glu Trp Val Glu Glu Tyr Asn Arg Gly Ala Pro Thr Leu Arg Lys  
290 295 300

Leu Met Glu Glu Ala Arg Thr His Pro Ile Glu Lys Val Gly Glu Glu  
305 310 315 320

Met Arg Lys Leu Leu Phe Gly Pro  
325

<210> SEQ ID NO 15  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Ralstonia solanacearum

<400> SEQUENCE: 15

Met Lys Val Phe Tyr Asp Lys Asp Ala Asp Leu Ser Leu Ile Lys Gly  
1 5 10 15

Lys Asn Val Thr Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala  
20 25 30

Leu Asn Leu Asn Asp Ser Gly Val Lys Val Thr Val Gly Leu Arg Lys  
35 40 45

Asn Gly Ala Ser Trp Asn Lys Ala Val Asn Ala Gly Leu Gln Val Lys  
50 55 60

Glu Val Ala Glu Ala Val Lys Asp Ala Asp Val Val Met Ile Leu Leu  
65 70 75 80

Pro Asp Glu Gln Ile Ala Asp Val Tyr Lys Asn Glu Val His Gly Asn  
85 90 95

Ile Lys Gln Gly Ala Ala Leu Ala Phe Ala His Gly Phe Asn Val His  
100 105 110

Tyr Gly Ala Val Ile Pro Arg Ala Asp Leu Asp Val Ile Met Val Ala  
115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Gly Thr Tyr Ala Gln Gly Gly  
130 135 140

Gly Val Pro His Leu Ile Ala Val His Gln Asp Lys Ser Gly Ser Ala  
145 150 155 160

Arg Asp Ile Ala Leu Ser Tyr Ala Thr Ala Asn Gly Gly Gly Arg Ala  
165 170 175

Gly Ile Ile Glu Thr Asn Phe Arg Glu Glu Thr Glu Thr Asp Leu Phe  
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Ile Lys Ala  
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Ile Tyr Glu  
225 230 235 240

Gly Gly Ile Gly Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
245 250 255

Gly Glu Tyr Val Thr Gly Pro Arg Val Val Thr Ala Glu Thr Lys Gln  
260 265 270

Ala Met Lys Gln Cys Leu His Asp Ile Gln Thr Gly Glu Tyr Ala Lys  
275 280 285

Ser Phe Leu Leu Glu Asn Lys Ala Gly Ala Pro Thr Leu Ile Ser Arg  
290 295 300

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Arg Arg Leu Thr Ala Asp His Gln Ile Glu Gln Val Gly Ala Lys Leu  
 305 310 315 320

Arg Ala Met Met Pro Trp Ile Ala Lys Asn Lys Leu Val Asp Gln Ser  
 325 330 335

Lys Asn

<210> SEQ ID NO 16  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Pseudomonas aeruginosa

&lt;400&gt; SEQUENCE: 16

Met Arg Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala  
 20 25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Arg Ser  
 35 40 45

Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Ala  
 50 55 60

Asp Val Lys Thr Ala Val Ala Ala Ala Asp Val Val Met Ile Leu Thr  
 65 70 75 80

Pro Asp Glu Phe Gln Gly Arg Leu Tyr Lys Glu Glu Ile Glu Pro Asn  
 85 90 95

Leu Lys Lys Gly Ala Thr Leu Ala Phe Ala His Gly Phe Ser Ile His  
 100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Cys Gly Val Gly Gly Gly Arg Thr  
 165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Cys Val Glu Leu Val Lys Ala  
 195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Ala  
 260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285

Met Phe Ile Thr Glu Gly Ala Ala Asn Tyr Pro Ser Met Thr Ala Tyr  
 290 295 300

Arg Arg Asn Asn Ala Ala His Pro Ile Glu Gln Ile Gly Glu Lys Leu  
 305 310 315 320

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Arg Ala Met Met Pro Trp Ile Ala Ala Asn Lys Ile Val Asp Lys Ser  
 325 330 335

Lys Asn

<210> SEQ ID NO 17  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Pseudomonas fluorescens

<400> SEQUENCE: 17

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Arg Lys  
 35 40 45

Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Thr  
 50 55 60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr  
 65 70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
 85 90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
 100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr  
 165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
 195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
 260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
 290 295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu  
 305 310 315 320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala  
 325 330 335



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Lys Asn

&lt;210&gt; SEQ ID NO 18

&lt;211&gt; LENGTH: 338

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Spinacia oleracea

&lt;400&gt; SEQUENCE: 18

Met Arg Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala  
 20 25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Arg Ser  
 35 40 45

Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Ala  
 50 55 60

Asp Val Lys Thr Ala Val Ala Ala Ala Asp Val Val Met Ile Leu Thr  
 65 70 75 80

Pro Asp Glu Phe Gln Gly Arg Leu Tyr Lys Glu Glu Ile Glu Pro Asn  
 85 90 95

Leu Lys Lys Gly Ala Thr Leu Ala Phe Ala His Gly Phe Ser Ile His  
 100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Cys Gly Val Gly Gly Gly Arg Thr  
 165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Cys Val Glu Leu Val Lys Ala  
 195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Ala  
 260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285

Met Phe Ile Thr Glu Gly Ala Ala Asn Tyr Pro Ser Met Thr Ala Tyr  
 290 295 300

Arg Arg Asn Asn Ala Ala His Pro Ile Glu Gln Ile Gly Glu Lys Leu  
 305 310 315 320

Arg Ala Met Met Pro Trp Ile Ala Ala Asn Lys Ile Val Asp Lys Ser  
 325 330 335

Lys Asn

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<210> SEQ ID NO 19
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorescens

<400> SEQUENCE: 19

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly
 1                               5 10 15

Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala
20                               25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys
35                               40 45

Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr
50                               55 60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr
65                               70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn
85                               90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His
100                              105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala
115                              120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly
130                              135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala
145                              150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Ala Val Gly Gly Gly Arg Thr
165                              170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe
180                              185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala
195                              200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
210                              215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225                              230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr
245                              250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln
260                              265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys
275                              280 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys
290                              295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu
305                              310 315 320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala
325                              330 335

Lys Asn

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<210> SEQ ID NO 20
<211> LENGTH: 338

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<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorescens

<400> SEQUENCE: 20

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly
 1                               5 10 15
Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala
20                               25 30
Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys
35                               40 45
Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr
50                               55 60
Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr
65                               70 75 80
Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn
85                               90 95
Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His
100                              105 110
Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala
115                              120 125
Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly
130                              135 140
Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala
145                              150 155 160
Lys Asn Val Ala Leu Ser Tyr Ala Ala Ala Val Gly Gly Gly Arg Thr
165                              170 175
Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe
180                              185 190
Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala
195                              200 205
Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
210                              215 220
Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225                              230 235 240
Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr
245                              250 255
Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln
260                              265 270
Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys
275                              280 285
Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys
290                              295 300
Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu
305                              310 315 320
Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala
325                              330 335

Lys Asn

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<210> SEQ ID NO 21
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:

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<223> OTHER INFORMATION: primer pBAD 266

<400> SEQUENCE: 21

ctctctactg tttctccata cccg 24

<210> SEQ ID NO 22

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: primer PF5- 53Mt

<220> FEATURE:

<221> NAME/KEY: misc\_feature

<222> LOCATION: (26)..(27)

<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 22

caagccgtgg gcttcagcct tggcknn 27

<210> SEQ ID NO 23

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: primer pBAD 866

<400> SEQUENCE: 23

cggtttcagt ctgctcctg aag 23

<210> SEQ ID NO 24

<211> LENGTH: 338

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: synthetic construct mutant ilcV

<400> SEQUENCE: 24

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
1 5 10 15

Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala  
20 25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys  
35 40 45

Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr  
50 55 60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr  
65 70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
85 90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Val Ser Gly Asn Ala  
145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr  
165 170 175

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Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
275 280 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
290 295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu  
305 310 315 320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala  
325 330 335

Lys Asn

<210> SEQ ID NO 25  
<211> LENGTH: 338  
<212> TYPE: PRT  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: synthetic construct mutant ilcV

&lt;400&gt; SEQUENCE: 25

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
1 5 10 15

Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala  
20 25 30

Leu Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys  
35 40 45

Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr  
50 55 60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr  
65 70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
85 90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala  
145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr  
165 170 175

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Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
 195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
 260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
 290 295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu  
 305 310 315 320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala  
 325 330 335

Lys Asn

<210> SEQ ID NO 26  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: a KARI mutant

&lt;400&gt; SEQUENCE: 26

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15

Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30

Leu Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys  
 35 40 45

Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr  
 50 55 60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr  
 65 70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
 85 90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
 100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Val Ser Gly Asn Ala  
 145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr  
 165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe

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180                    185                    190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
195                    200                    205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
210                    215                    220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
225                    230                    235                    240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
245                    250                    255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
260                    265                    270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
275                    280                    285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
290                    295                    300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu  
305                    310                    315                    320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala  
325                    330                    335

Lys Asn

<210> SEQ ID NO 27  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct mutant ilcV

<400> SEQUENCE: 27

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
1                    5                    10                    15

Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala  
20                    25                    30

Leu Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys  
35                    40                    45

Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr  
50                    55                    60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr  
65                    70                    75                    80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
85                    90                    95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
100                    105                    110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
115                    120                    125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
130                    135                    140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala  
145                    150                    155                    160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Ala Val Gly Gly Gly Arg Thr  
165                    170                    175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
180                    185                    190

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Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
275 280 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
290 295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu  
305 310 315 320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala  
325 330 335

Lys Asn

<210> SEQ ID NO 28  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic construct mutant ilcV

<400> SEQUENCE: 28

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
20 25 30

Leu Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys  
35 40 45

Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr  
50 55 60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Ile  
65 70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
85 90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Val Ser Gly Asn Ala  
145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Ala Val Gly Gly Gly Arg Thr  
165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
180 185 190



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Gly	Glu	Gln	Ala	Val	Leu	Cys	Gly	Gly	Thr	Val	Glu	Leu	Val	Lys	Ala
195					200					205					
Gly	Phe	Glu	Thr	Leu	Val	Glu	Ala	Gly	Tyr	Ala	Pro	Glu	Met	Ala	Tyr
210				215						220					
Phe	Glu	Cys	Leu	His	Glu	Leu	Lys	Leu	Ile	Val	Asp	Leu	Met	Tyr	Glu
225				230						235				240	
Gly	Gly	Ile	Ala	Asn	Met	Asn	Tyr	Ser	Ile	Ser	Asn	Asn	Ala	Glu	Tyr
245				250						255					
Gly	Glu	Tyr	Val	Thr	Gly	Pro	Glu	Val	Ile	Asn	Ala	Glu	Ser	Arg	Gln
260					265					270					
Ala	Met	Arg	Asn	Ala	Leu	Lys	Arg	Ile	Gln	Asp	Gly	Glu	Tyr	Ala	Lys
275				280						285					
Met	Phe	Ile	Ser	Glu	Gly	Ala	Thr	Gly	Tyr	Pro	Ser	Met	Thr	Ala	Lys
290				295						300					
Arg	Arg	Asn	Asn	Ala	Ala	His	Gly	Ile	Glu	Ile	Ile	Gly	Glu	Gln	Leu
305				310						315				320	
Arg	Ser	Met	Met	Pro	Trp	Ile	Gly	Ala	Asn	Lys	Ile	Val	Asp	Lys	Ala
325				330						335					

Lys Asn

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<210> SEQ ID NO 29
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: Xaa = Tyr or Phe
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (33)..(33)
<223> OTHER INFORMATION: Xaa = Cys or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (47)..(47)
<223> OTHER INFORMATION: Xaa = Arg or Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (50)..(50)
<223> OTHER INFORMATION: Xaa = Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (52)..(52)
<223> OTHER INFORMATION: Xaa = Thr or Asp
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (53)..(53)
<223> OTHER INFORMATION: Xaa = Val or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (61)..(61)
<223> OTHER INFORMATION: Xaa = Leu or Phe
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (80)..(80)
<223> OTHER INFORMATION: Xaa = Thr or Iso
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (156)..(156)
<223> OTHER INFORMATION: Xaa = Ala or Val
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (170)..(170)
<223> OTHER INFORMATION: Xaa = Gly or Ala

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&lt;400&gt; SEQUENCE: 29

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Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly
 1           5           10           15
Lys Lys Val Ala Ile Ile Gly Xaa Gly Ser Gln Gly His Ala Gln Ala
20           25           30
Xaa Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Xaa Lys
35           40           45
Gly Xaa Ala Xaa Xaa Ala Lys Ala Glu Ala His Gly Xaa Lys Val Thr
50           55           60
Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Xaa
65           70           75           80
Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn
85           90           95
Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His
100          105          110
Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala
115          120          125
Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly
130          135          140
Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Xaa Ser Gly Asn Ala
145          150          155          160
Lys Asn Val Ala Leu Ser Tyr Ala Ala Xaa Val Gly Gly Gly Arg Thr
165          170          175
Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe
180          185          190
Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala
195          200          205
Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
210          215          220
Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225          230          235          240
Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr
245          250          255
Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln
260          265          270
Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys
275          280          285
Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys
290          295          300
Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu
305          310          315          320
Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala
325          330          335

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Lys Asn

&lt;210&gt; SEQ ID NO 30

&lt;211&gt; LENGTH: 331

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Natronomonas pharaanis

&lt;400&gt; SEQUENCE: 30

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Met Thr Asp Ala Thr Ile Tyr Tyr Asp Asp Asp Ala Glu Ser Thr Val
 1          5          10          15
Leu Asp Asp Lys Thr Val Ala Val Leu Gly Tyr Gly Ser Gln Gly His
20          25          30
Ala His Ala Gln Asn Leu Asp Asp Ser Gly Val Asp Val Val Val Gly
35          40          45
Leu Arg Glu Asp Ser Ser Ser Arg Ser Ala Ala Glu Ala Asp Gly Leu
50          55          60
Asp Val Ala Thr Pro Arg Gly Ala Ala Glu Gln Ala Asp Leu Val Ser
65          70          75          80
Val Leu Val Pro Asp Thr Val Gln Pro Ala Val Tyr Glu Gln Ile Glu
85          90          95
Asp Val Leu Gln Pro Gly Asp Thr Leu Gln Phe Ala His Gly Phe Asn
100         105         110
Ile His Tyr Gly Gln Ile Glu Pro Ser Glu Asp Val Asn Val Thr Met
115         120         125
Val Ala Pro Lys Ser Pro Gly His Leu Val Arg Arg Asn Tyr Glu Asn
130         135         140
Asp Glu Gly Thr Pro Gly Leu Leu Ala Val Tyr Gln Asp Pro Ser Gly
145         150         155         160
Glu Ala His Asp Leu Gly Leu Ala Tyr Ala Lys Ala Ile Gly Cys Thr
165         170         175
Arg Ala Gly Val Val Glu Thr Thr Phe Arg Glu Glu Thr Glu Thr Asp
180         185         190
Leu Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Val Thr Ser Leu Val
195         200         205
Lys Thr Gly Tyr Glu Thr Leu Val Asp Ala Gly Tyr Ser Pro Glu Met
210         215         220
Ala Tyr Phe Glu Cys Leu Asn Glu Leu Lys Leu Ile Val Asp Leu Met
225         230         235         240
Tyr Glu Gly Gly Asn Ser Glu Met Trp Asp Ser Val Ser Asp Thr Ala
245         250         255
Glu Tyr Gly Gly Leu Thr Arg Gly Asp Arg Ile Val Asp Asp His Ala
260         265         270
Arg Glu Lys Met Glu Glu Val Leu Glu Glu Val Gln Asn Gly Thr Phe
275         280         285
Ala Arg Glu Trp Ile Ser Glu Asn Gln Ala Gly Arg Pro Ser Tyr Lys
290         295         300
Gln Leu Arg Ala Ala Glu Lys Asn His Asp Ile Glu Ala Val Gly Glu
305         310         315         320
Asp Leu Arg Ala Leu Phe Ala Trp Gly Asp Asp
325         330

```

&lt;210&gt; SEQ ID NO 31

&lt;211&gt; LENGTH: 342

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus subtilis

&lt;400&gt; SEQUENCE: 31

```

Met Val Lys Val Tyr Tyr Asn Gly Asp Ile Lys Glu Asn Val Leu Ala
 1          5          10          15
Gly Lys Thr Val Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His
20          25          30

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Ala Leu Asn Leu Lys Glu Ser Gly Val Asp Val Ile Val Gly Val Arg  
35 40 45

Gln Gly Lys Ser Phe Thr Gln Ala Gln Glu Asp Gly His Lys Val Phe  
50 55 60

Ser Val Lys Glu Ala Ala Ala Gln Ala Glu Ile Ile Met Val Leu Leu  
65 70 75 80

Pro Asp Glu Gln Gln Gln Lys Val Tyr Glu Ala Glu Ile Lys Asp Glu  
85 90 95

Leu Thr Ala Gly Lys Ser Leu Val Phe Ala His Gly Phe Asn Val His  
100 105 110

Phe His Gln Ile Val Pro Pro Ala Asp Val Asp Val Phe Leu Val Ala  
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Tyr Glu Gln Gly Ala  
130 135 140

Gly Val Pro Ala Leu Phe Ala Ile Tyr Gln Asp Val Thr Gly Glu Ala  
145 150 155 160

Arg Asp Lys Ala Leu Ala Tyr Ala Lys Gly Ile Gly Gly Ala Arg Ala  
165 170 175

Gly Val Leu Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe  
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Ser Ala Leu Val Lys Ala  
195 200 205

Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr  
210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
225 230 235 240

Glu Gly Leu Ala Gly Met Arg Tyr Ser Ile Ser Asp Thr Ala Gln Trp  
245 250 255

Gly Asp Phe Val Ser Gly Pro Arg Val Val Asp Ala Lys Val Lys Glu  
260 265 270

Ser Met Lys Glu Val Leu Lys Asp Ile Gln Asn Gly Thr Phe Ala Lys  
275 280 285

Glu Trp Ile Val Glu Asn Gln Val Asn Arg Pro Arg Phe Asn Ala Ile  
290 295 300

Asn Ala Ser Glu Asn Glu His Gln Ile Glu Val Val Gly Arg Lys Leu  
305 310 315 320

Arg Glu Met Met Pro Phe Val Lys Gln Gly Lys Lys Lys Glu Ala Val  
325 330 335

Val Ser Val Ala Gln Asn  
340

&lt;210&gt; SEQ ID NO 32

&lt;211&gt; LENGTH: 338

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Corynebacterium glutamicum

&lt;400&gt; SEQUENCE: 32

Met Ala Ile Glu Leu Leu Tyr Asp Ala Asp Ala Asp Leu Ser Leu Ile  
1 5 10 15

Gln Gly Arg Lys Val Ala Ile Val Gly Tyr Gly Ser Gln Gly His Ala  
20 25 30

His Ser Gln Asn Leu Arg Asp Ser Gly Val Glu Val Val Ile Gly Leu

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35          40          45
Arg Glu Gly Ser Lys Ser Ala Glu Lys Ala Lys Glu Ala Gly Phe Glu
50          55          60
Val Lys Thr Thr Ala Glu Ala Ala Ala Trp Ala Asp Val Ile Met Leu
65          70          75          80
Leu Ala Pro Asp Thr Ser Gln Ala Glu Ile Phe Thr Asn Asp Ile Glu
85          90          95
Pro Asn Leu Asn Ala Gly Asp Ala Leu Leu Phe Gly His Gly Leu Asn
100         105         110
Ile His Phe Asp Leu Ile Lys Pro Ala Asp Asp Ile Ile Val Gly Met
115         120         125
Val Ala Pro Lys Gly Pro Gly His Leu Val Arg Arg Gln Phe Val Asp
130         135         140
Gly Lys Gly Val Pro Cys Leu Ile Ala Val Asp Gln Asp Pro Thr Gly
145         150         155         160
Thr Ala Gln Ala Leu Thr Leu Ser Tyr Ala Ala Ala Ile Gly Gly Ala
165         170         175
Arg Ala Gly Val Ile Pro Thr Thr Phe Glu Ala Glu Thr Val Thr Asp
180         185         190
Leu Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Glu Glu Leu Val
195         200         205
Lys Val Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Glu Pro Glu Met
210         215         220
Ala Tyr Phe Glu Val Leu His Glu Leu Lys Leu Ile Val Asp Leu Met
225         230         235         240
Phe Glu Gly Gly Ile Ser Asn Met Asn Tyr Ser Val Ser Asp Thr Ala
245         250         255
Glu Phe Gly Gly Tyr Leu Ser Gly Pro Arg Val Ile Asp Ala Asp Thr
260         265         270
Lys Ser Arg Met Lys Asp Ile Leu Thr Asp Ile Gln Asp Gly Thr Phe
275         280         285
Thr Lys Arg Leu Ile Ala Asn Val Glu Asn Gly Asn Thr Glu Leu Glu
290         295         300
Gly Leu Arg Ala Ser Tyr Asn Asn His Pro Ile Glu Glu Thr Gly Ala
305         310         315         320
Lys Leu Arg Asp Leu Met Ser Trp Val Lys Val Asp Ala Arg Ala Glu
325         330         335
Thr Ala

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<210> SEQ ID NO 33
<211> LENGTH: 339
<212> TYPE: PRT
<213> ORGANISM: Phaeospririllum molischianum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (310)..(310)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<400> SEQUENCE: 33

```

```

Met Arg Val Tyr Tyr Asp Arg Asp Ala Asp Val Asn Leu Ile Lys Ser
1          5          10          15
Lys Lys Val Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His Val
20         25          30

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Leu Asn Leu Arg Asp Ser Gly Val Lys Asp Val Ala Val Ala Leu Arg  
 35 40 45  
 Pro Gly Ser Ala Ser Ile Lys Lys Ala Glu Ala Glu Gly Leu Lys Val  
 50 55 60  
 Leu Thr Pro Ala Glu Ala Ala Ala Trp Ala Asp Val Val Met Ile Leu  
 65 70 75 80  
 Thr Pro Asp Glu Leu Gln Ala Asp Leu Tyr Lys Ser Glu Leu Ala Ala  
 85 90 95  
 Asn Leu Lys Pro Gly Ala Ala Leu Val Phe Ala His Gly Leu Ala Ile  
 100 105 110  
 His Phe Lys Leu Ile Glu Ala Arg Ala Asp Leu Asp Val Phe Met Val  
 115 120 125  
 Ala Pro Lys Gly Pro Gly His Thr Val Arg Gly Glu Tyr Leu Lys Gly  
 130 135 140  
 Gly Gly Val Pro Cys Leu Val Ala Val Ala Gln Asn Pro Thr Gly Asn  
 145 150 155 160  
 Ala Leu Glu Leu Ala Leu Ser Tyr Ala Ser Ala Ile Gly Gly Gly Arg  
 165 170 175  
 Ser Gly Ile Ile Glu Thr Thr Phe Arg Glu Glu Cys Glu Thr Asp Leu  
 180 185 190  
 Phe Gly Glu Gln Val Val Leu Cys Gly Gly Leu Ser Lys Leu Ile Gln  
 195 200 205  
 Tyr Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala  
 210 215 220  
 Tyr Phe Glu Cys Leu His Glu Val Lys Leu Ile Val Asp Leu Ile Tyr  
 225 230 235 240  
 Glu Gly Gly Ile Ala Asn Met Arg Tyr Ser Ile Ser Asn Thr Ala Glu  
 245 250 255  
 Tyr Gly Asp Tyr Val Thr Gly Ser Arg Ile Ile Thr Glu Ala Thr Lys  
 260 265 270  
 Ala Glu Met Lys Arg Val Leu Ala Asp Ile Gln Ser Gly Arg Phe Val  
 275 280 285  
 Arg Asp Trp Met Leu Glu Cys Lys Ala Gly Gln Pro Ser Phe Lys Ala  
 290 295 300  
 Thr Arg Arg Ile Gln Xaa Glu His Val Ile Glu Val Val Gly Glu Lys  
 305 310 315 320  
 Leu Arg Gly Met Met Pro Trp Ile Ser Lys Asn Lys Leu Val Asp Lys  
 325 330 335  
 Ala Arg Asn

&lt;210&gt; SEQ ID NO 34

&lt;211&gt; LENGTH: 339

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Zymomonas mobilis*

&lt;400&gt; SEQUENCE: 34

Met Lys Val Tyr Tyr Asp Ser Asp Ala Asp Leu Gly Leu Ile Lys Ser  
 1 5 10 15  
 Lys Lys Ile Ala Ile Leu Gly Tyr Gly Ser Gln Gly His Ala His Ala  
 20 25 30  
 Gln Asn Leu Arg Asp Ser Gly Val Ala Glu Val Ala Ile Ala Leu Arg  
 35 40 45

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Pro Asp Ser Ala Ser Val Lys Lys Ala Gln Asp Ala Gly Phe Lys Val  
 50 55 60  
 Leu Thr Asn Ala Glu Ala Ala Lys Trp Ala Asp Ile Leu Met Ile Leu  
 65 70 75 80  
 Ala Pro Asp Glu His Gln Ala Ala Ile Tyr Ala Glu Asp Leu Lys Asp  
 85 90 95  
 Asn Leu Arg Pro Gly Ser Ala Ile Ala Phe Ala His Gly Leu Asn Ile  
 100 105 110  
 His Phe Gly Leu Ile Glu Pro Arg Lys Asp Ile Asp Val Phe Met Ile  
 115 120 125  
 Ala Pro Lys Gly Pro Gly His Thr Val Arg Ser Glu Tyr Val Arg Gly  
 130 135 140  
 Gly Gly Val Pro Cys Leu Val Ala Val Asp Gln Asp Ala Ser Gly Asn  
 145 150 155 160  
 Ala His Asp Ile Ala Leu Ala Tyr Ala Ser Gly Ile Gly Gly Gly Arg  
 165 170 175  
 Ser Gly Val Ile Glu Thr Thr Phe Arg Glu Glu Val Glu Thr Asp Leu  
 180 185 190  
 Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile Thr  
 195 200 205  
 Ala Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Ala Pro Glu Met Ala  
 210 215 220  
 Phe Phe Glu Cys Met His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr  
 225 230 235 240  
 Glu Ala Gly Ile Ala Asn Met Arg Tyr Ser Ile Ser Asn Thr Ala Glu  
 245 250 255  
 Tyr Gly Asp Ile Val Ser Gly Pro Arg Val Ile Asn Glu Glu Ser Lys  
 260 265 270  
 Lys Ala Met Lys Ala Ile Leu Asp Asp Ile Gln Ser Gly Arg Phe Val  
 275 280 285  
 Ser Lys Phe Val Leu Asp Asn Arg Ala Gly Gln Pro Glu Leu Lys Ala  
 290 295 300  
 Ala Arg Lys Arg Met Ala Ala His Pro Ile Glu Gln Val Gly Ala Arg  
 305 310 315 320  
 Leu Arg Lys Met Met Pro Trp Ile Ala Ser Asn Lys Leu Val Asp Lys  
 325 330 335  
 Ala Arg Asn

<210> SEQ ID NO 35  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Alkalilimnicola ehrlichei

<400> SEQUENCE: 35

Met Gln Val Tyr Tyr Asp Lys Asp Ala Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15  
 Lys Lys Val Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala  
 20 25 30  
 Asn Asn Leu Lys Glu Ser Gly Val Asp Val Val Val Gly Leu Arg Glu  
 35 40 45  
 Gly Ser Ser Ser Ala Ala Lys Ala Gln Lys Ala Gly Leu Ala Val Ala  
 50 55 60

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Ser Ile Glu Asp Ala Ala Ala Gln Ala Asp Val Val Met Ile Leu Ala
65          70          75          80

Pro Asp Glu His Gln Ala Val Ile Tyr His Asn Gln Ile Ala Pro Asn
85          90          95

Val Lys Pro Gly Ala Ala Ile Ala Phe Ala His Gly Phe Asn Ile His
100         105         110

Phe Gly Gln Ile Gln Pro Ala Ala Asp Leu Asp Val Ile Met Val Ala
115         120         125

Pro Lys Gly Pro Gly His Leu Val Arg Ser Thr Tyr Val Glu Gly Gly
130         135         140

Gly Val Pro Ser Leu Ile Ala Ile His Gln Asp Ala Thr Gly Lys Ala
145         150         155         160

Lys Asp Ile Ala Leu Ser Tyr Ala Ser Ala Asn Gly Gly Gly Arg Ala
165         170         175

Gly Val Ile Glu Thr Ser Phe Arg Glu Glu Thr Glu Thr Asp Leu Phe
180         185         190

Gly Glu Gln Ala Val Leu Cys Gly Gly Ile Thr Ser Leu Ile Gln Ala
195         200         205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
210         215         220

Phe Glu Cys Leu His Glu Thr Lys Leu Ile Val Asp Leu Leu Tyr Gln
225         230         235         240

Gly Gly Ile Ala Asn Met Arg Tyr Ser Ile Ser Asn Thr Ala Glu Tyr
245         250         255

Gly Asp Phe Thr Arg Gly Pro Arg Val Ile Asn Glu Glu Ser Arg Glu
260         265         270

Ala Met Arg Glu Ile Leu Ala Glu Ile Gln Glu Gly Glu Phe Ala Arg
275         280         285

Glu Phe Val Leu Glu Asn Gln Ala Gly Cys Pro Thr Leu Thr Ala Arg
290         295         300

Arg Arg Leu Ala Ala Glu His Glu Ile Glu Val Val Gly Glu Arg Leu
305         310         315         320

Arg Gly Met Met Pro Trp Ile Asn Ala Asn Lys Leu Val Asp Lys Asp
325         330         335

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Lys Asn

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<210> SEQ ID NO 36
<211> LENGTH: 340
<212> TYPE: PRT
<213> ORGANISM: Campylobacter lari

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&lt;400&gt; SEQUENCE: 36

```

Met Ala Val Ser Ile Tyr Tyr Asp Lys Asp Cys Asp Ile Asn Leu Ile
1          5          10         15

Lys Ser Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala
20         25         30

His Ala Met Asn Leu Arg Asp Ser Gly Val Glu Val Ile Ile Gly Leu
35         40         45

Lys Glu Gly Gly Gln Ser Trp Ala Lys Ala Gln Lys Ala Asn Phe Ile
50         55         60

Val Lys Ser Val Lys Glu Ala Thr Lys Glu Ala Asp Leu Ile Met Ile
65         70         75         80

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Leu Ala Pro Asp Glu Ile Gln Ser Glu Ile Phe Asn Glu Glu Ile Lys  
 85 90 95  
 Pro Glu Leu Lys Ala Gly Lys Thr Leu Ala Phe Ala His Gly Phe Asn  
 100 105 110  
 Ile His Tyr Gly Gln Ile Val Ala Pro Lys Gly Ile Asp Val Ile Met  
 115 120 125  
 Ile Ala Pro Lys Ala Pro Gly His Thr Val Arg His Glu Phe Ser Ile  
 130 135 140  
 Gly Gly Gly Thr Pro Cys Leu Ile Ala Ile His Gln Asp Glu Ser Lys  
 145 150 155 160  
 Asn Ala Lys Asn Leu Ala Leu Ser Tyr Ala Ser Ala Ile Gly Gly Gly  
 165 170 175  
 Arg Thr Gly Ile Ile Glu Thr Thr Phe Lys Ala Glu Thr Glu Thr Asp  
 180 185 190  
 Leu Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Ser Ala Leu Ile  
 195 200 205  
 Gln Ala Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Glu Pro Glu Met  
 210 215 220  
 Ala Tyr Phe Glu Cys Leu His Glu Met Lys Leu Ile Val Asp Leu Ile  
 225 230 235 240  
 Tyr Gln Gly Gly Ile Ala Asp Met Arg Tyr Ser Val Ser Asn Thr Ala  
 245 250 255  
 Glu Tyr Gly Asp Tyr Ile Thr Gly Pro Lys Ile Ile Thr Lys Glu Thr  
 260 265 270  
 Lys Glu Ala Met Lys Gly Val Leu Lys Asp Ile Gln Asn Gly Ser Phe  
 275 280 285  
 Ala Lys Asp Phe Ile Leu Glu Arg Arg Ala Asn Phe Ala Arg Met His  
 290 295 300  
 Ala Glu Arg Lys Leu Met Asn Asp Ser Leu Ile Glu Lys Thr Gly Arg  
 305 310 315 320  
 Glu Leu Arg Ala Met Met Pro Trp Ile Ser Ala Lys Lys Leu Val Asp  
 325 330 335  
 Lys Asp Lys Asn  
 340

&lt;210&gt; SEQ ID NO 37

&lt;211&gt; LENGTH: 338

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Marinobacter aquaeolei

&lt;400&gt; SEQUENCE: 37

Met Gln Val Tyr Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15  
 Lys Lys Val Ala Ile Leu Gly Phe Gly Ser Gln Gly His Ala His Ala  
 20 25 30  
 Cys Asn Leu Lys Asp Ser Gly Val Asp Val Val Val Gly Leu Arg Ala  
 35 40 45  
 Gly Ser Ser Ser Ile Ala Lys Ala Glu Ala Tyr Gly Leu Lys Thr Ser  
 50 55 60  
 Asp Val Ala Ser Ala Val Ala Ser Ala Asp Val Val Met Val Leu Thr  
 65 70 75 80  
 Pro Asp Glu Phe Gln Ala Gln Leu Tyr Arg Glu Glu Ile Glu Pro Asn  
 85 90 95

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Leu Lys Gln Gly Ala Thr Leu Ala Phe Ala His Gly Phe Ala Ile His  
 100 105 110  
 Tyr Asn Gln Ile Val Pro Arg Lys Asp Leu Asp Val Ile Met Val Ala  
 115 120 125  
 Pro Lys Ala Pro Gly His Thr Val Arg Thr Glu Phe Thr Lys Gly Gly  
 130 135 140  
 Gly Ile Pro Asp Leu Ile Ala Ile Phe Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160  
 Lys Asn Val Ala Leu Ser Tyr Ala Ser Gly Ile Gly Gly Gly Arg Thr  
 165 170 175  
 Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Ala Val Glu Leu Val Lys Ala  
 195 200 205  
 Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240  
 Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255  
 Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Glu Gln Ser Arg Glu  
 260 265 270  
 Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Ser Gly Glu Tyr Ala Lys  
 275 280 285  
 Met Phe Ile Ser Glu Gly Ala Leu Asn Tyr Pro Ser Met Thr Ala Arg  
 290 295 300  
 Arg Arg Gln Asn Ala Ala His Glu Ile Glu Thr Val Gly Glu Lys Leu  
 305 310 315 320  
 Arg Ser Met Met Pro Trp Ile Ser Ala Asn Lys Ile Val Asp Lys Asp  
 325 330 335  
 Lys Asn

<210> SEQ ID NO 38  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Psychrobacter arcticus

<400> SEQUENCE: 38

Met Asn Val Tyr Tyr Asp Lys Asp Cys Asp Leu Ser Ile Val Gln Gly  
 1 5 10 15  
 Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala  
 20 25 30  
 Leu Asn Leu Gln Asp Ser Asn Val Asp Val Thr Val Gly Leu Arg Ala  
 35 40 45  
 Asp Ser Gly Ser Trp Lys Lys Ala Glu Asn Ala Gly Leu Lys Val Ala  
 50 55 60  
 Glu Val Glu Glu Ala Val Lys Ala Ala Asp Ile Ile Met Ile Leu Thr  
 65 70 75 80  
 Pro Asp Glu Phe Gln Lys Glu Leu Tyr Asn Asp Val Ile Glu Pro Asn  
 85 90 95  
 Ile Lys Gln Gly Ala Thr Leu Ala Phe Ala His Gly Phe Ala Ile His  
 100 105 110

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Tyr Asn Gln Val Ile Pro Arg Ser Asp Leu Asp Val Ile Met Val Ala  
 115 120 125  
 Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Ala Lys Gly Gly  
 130 135 140  
 Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Gln Ala  
 145 150 155 160  
 Lys Gln Leu Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Ser  
 165 170 175  
 Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Ala Val Glu Leu Val Lys Met  
 195 200 205  
 Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240  
 Gly Gly Ile Ala Asp Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255  
 Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Glu Gln Ser Arg Glu  
 260 265 270  
 Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Ser Gly Glu Tyr Ala Lys  
 275 280 285  
 Met Phe Ile Ser Glu Gly Ala Thr Asn Tyr Pro Ser Met Thr Ala Arg  
 290 295 300  
 Arg Arg Asn Asn Ala Glu His Gln Ile Glu Ile Thr Gly Ala Lys Leu  
 305 310 315 320  
 Arg Gly Met Met Pro Trp Ile Gly Gly Asn Lys Ile Ile Asp Lys Asp  
 325 330 335

Lys Asn

<210> SEQ ID NO 39  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: *Hahella chejuensis*

&lt;400&gt; SEQUENCE: 39

Met Gln Val Tyr Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15  
 Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala  
 20 25 30  
 Asn Asn Leu Lys Asp Ser Gly Val Asp Val Cys Val Gly Leu Arg Lys  
 35 40 45  
 Gly Ser Gly Ser Trp Ala Lys Ala Glu Asn Ala Gly Leu Ala Val Lys  
 50 55 60  
 Glu Val Ala Glu Ala Val Ala Gly Ala Asp Val Val Met Ile Leu Thr  
 65 70 75 80  
 Pro Asp Glu Phe Gln Ala Gln Leu Tyr Lys Ser Glu Ile Glu Pro Asn  
 85 90 95  
 Leu Lys Ser Gly Ala Thr Leu Ala Phe Ala His Gly Phe Ser Ile His  
 100 105 110  
 Tyr Asn Gln Ile Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125

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Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140  
 Gly Ile Pro Asp Leu Ile Ala Ile Phe Gln Asp Ala Ser Gly Ser Ala  
 145 150 155 160  
 Lys Asp Leu Ala Leu Ser Tyr Ala Ser Gly Val Gly Gly Gly Arg Thr  
 165 170 175  
 Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Ala Val Glu Leu Val Lys Ala  
 195 200 205  
 Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240  
 Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255  
 Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Asp Gln Ser Arg Ala  
 260 265 270  
 Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285  
 Met Phe Ile Ala Glu Gly Ala His Asn Tyr Pro Ser Met Thr Ala Tyr  
 290 295 300  
 Arg Arg Asn Asn Ala Ala His Pro Ile Glu Gln Val Gly Glu Lys Leu  
 305 310 315 320  
 Arg Ser Met Met Pro Trp Ile Ala Ser Asn Lys Ile Val Asp Lys Ser  
 325 330 335  
 Lys Asn

<210> SEQ ID NO 40  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Thiobacillus denitrificans

<400> SEQUENCE: 40

Met Lys Val Tyr Tyr Asp Lys Asp Ala Asp Leu Ser Leu Ile Lys Gln  
 1 5 10 15  
 Arg Lys Val Ala Ile Val Gly Tyr Gly Ser Gln Gly His Ala His Ala  
 20 25 30  
 Asn Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Ala Leu Arg Pro  
 35 40 45  
 Gly Ser Ala Ser Ala Lys Lys Ala Glu Asn Ala Gly Leu Thr Val Lys  
 50 55 60  
 Ser Val Pro Glu Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr  
 65 70 75 80  
 Pro Asp Glu Phe Gln Ser Arg Leu Tyr Arg Asp Glu Ile Glu Pro Asn  
 85 90 95  
 Ile Lys Gln Gly Ala Thr Leu Ala Phe Ala His Gly Phe Ser Ile His  
 100 105 110  
 Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125  
 Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140

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Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Lys Ala  
 145 150 155 160  
 Lys Glu Thr Ala Leu Ser Tyr Ala Ser Ala Ile Gly Gly Gly Arg Thr  
 165 170 175  
 Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Ala Val Glu Leu Val Lys Ala  
 195 200 205  
 Gly Phe Asp Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240  
 Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255  
 Gly Glu Tyr Val Thr Gly Val Lys Val Ile Asn Glu Gln Ser Arg Ala  
 260 265 270  
 Ala Met Lys Glu Cys Leu Ala Asn Ile Gln Asn Gly Ala Tyr Ala Lys  
 275 280 285  
 Arg Phe Ile Leu Glu Gly Gln Ala Asn Tyr Pro Glu Met Thr Ala Trp  
 290 295 300  
 Arg Arg Asn Asn Ala Ala His Gln Ile Glu Val Val Gly Ala Lys Leu  
 305 310 315 320  
 Arg Ser Met Met Pro Trp Ile Ala Ala Asn Lys Leu Val Asp His Ser  
 325 330 335

Lys Asn

<210> SEQ ID NO 41  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Azotobacter vinelandii

&lt;400&gt; SEQUENCE: 41

Met Lys Val Tyr Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Ser  
 1 5 10 15  
 Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala  
 20 25 30  
 Cys Asn Leu Lys Asp Ser Gly Val Asp Val Tyr Val Gly Leu Arg Ala  
 35 40 45  
 Gly Ser Ala Ser Val Ala Lys Ala Glu Ala His Gly Leu Thr Val Lys  
 50 55 60  
 Ser Val Lys Asp Ala Val Ala Ala Ala Asp Val Val Met Ile Leu Thr  
 65 70 75 80  
 Pro Asp Glu Phe Gln Gly Arg Leu Tyr Lys Asp Glu Ile Glu Pro Asn  
 85 90 95  
 Leu Lys Lys Gly Ala Thr Leu Ala Phe Ala His Gly Phe Ser Ile His  
 100 105 110  
 Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125  
 Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Arg Gly Gly  
 130 135 140  
 Gly Ile Pro Asp Leu Ile Ala Val Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160

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Lys Asn Leu Ala Leu Ser Tyr Ala Cys Gly Val Gly Gly Gly Arg Thr  
165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Cys Val Glu Leu Val Lys Ala  
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Phe Glu  
225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Glu Gln Ser Arg Gln  
260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
275 280 285

Met Phe Ile Thr Glu Gly Ala Ala Asn Tyr Pro Ser Met Thr Ala Tyr  
290 295 300

Arg Arg Asn Asn Ala Ala His Gln Ile Glu Val Val Gly Glu Lys Leu  
305 310 315 320

Arg Thr Met Met Pro Trp Ile Ala Ala Asn Lys Ile Val Asp Lys Thr  
325 330 335

Lys Asn

<210> SEQ ID NO 42  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Pseudomonas syringae

&lt;400&gt; SEQUENCE: 42

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
20 25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Arg Lys  
35 40 45

Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Thr  
50 55 60

Asp Val Ala Ser Ala Val Ala Ala Ala Asp Leu Val Met Ile Leu Thr  
65 70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Val Glu Pro Asn  
85 90 95

Leu Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Thr Glu Phe Val Lys Gly Gly  
130 135 140

Gly Ile Pro Asp Leu Ile Ala Val Tyr Gln Asp Ala Ser Gly Asn Ala  
145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ser Gly Val Gly Gly Gly Arg Thr  
165 170 175

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Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
 195 200 205  
 Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240  
 Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255  
 Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
 260 265 270  
 Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285  
 Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
 290 295 300  
 Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Lys Leu  
 305 310 315 320  
 Arg Ser Met Met Pro Trp Ile Ala Ala Asn Lys Ile Val Asp Lys Asp  
 325 330 335  
 Lys Asn

<210> SEQ ID NO 43  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Pseudomonas syringae

<400> SEQUENCE: 43

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15  
 Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30  
 Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Arg Lys  
 35 40 45  
 Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Thr  
 50 55 60  
 Asp Val Ala Ser Ala Val Ala Ala Ala Asp Leu Val Met Ile Leu Thr  
 65 70 75 80  
 Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Val Glu Pro Asn  
 85 90 95  
 Leu Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
 100 105 110  
 Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125  
 Pro Lys Ala Pro Gly His Thr Val Arg Thr Glu Phe Val Lys Gly Gly  
 130 135 140  
 Gly Ile Pro Asp Leu Ile Ala Val Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160  
 Lys Asn Val Ala Leu Ser Tyr Ala Ser Gly Val Gly Gly Gly Arg Thr  
 165 170 175  
 Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190

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Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
 195 200 205  
 Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240  
 Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255  
 Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
 260 265 270  
 Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285  
 Met Phe Ile Thr Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
 290 295 300  
 Arg Arg Asn Asn Ala Glu His Gly Ile Glu Val Ile Gly Glu Lys Leu  
 305 310 315 320  
 Arg Ser Met Met Pro Trp Ile Ala Ala Asn Lys Ile Val Asp Lys Asp  
 325 330 335  
 Lys Asn

<210> SEQ ID NO 44  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Pseudomonas putida

<400> SEQUENCE: 44

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15  
 Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30  
 Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Arg Lys  
 35 40 45  
 Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Ala  
 50 55 60  
 Asp Val Ala Thr Ala Val Ala Ala Ala Asp Leu Val Met Ile Leu Thr  
 65 70 75 80  
 Pro Asp Glu Phe Gln Gly Ala Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
 85 90 95  
 Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ser Ile His  
 100 105 110  
 Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125  
 Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140  
 Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160  
 Lys Asn Val Ala Leu Ser Tyr Ala Ser Gly Val Gly Gly Gly Arg Thr  
 165 170 175  
 Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
 195 200 205



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Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Glu Glu Ser Arg Lys  
 260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285

Met Phe Ile Ser Glu Gly Ala Thr Asn Tyr Pro Ser Met Thr Ala Lys  
 290 295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu  
 305 310 315 320

Arg Ser Met Met Pro Trp Ile Ser Ala Asn Lys Ile Val Asp Lys Thr  
 325 330 335

Lys Asn

<210> SEQ ID NO 45  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: Pseudomonas entomophila

<400> SEQUENCE: 45

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Ile Gly Leu Arg Lys  
 35 40 45

Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Thr  
 50 55 60

Asp Val Ala Thr Ala Val Ala Ala Ala Asp Leu Val Met Ile Leu Thr  
 65 70 75 80

Pro Asp Glu Phe Gln Gly Gln Leu Tyr Lys Gln Glu Ile Glu Pro Asn  
 85 90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
 100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ser Gly Val Gly Gly Gly Arg Thr  
 165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
 195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220

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Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240  
 Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255  
 Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Glu Glu Ser Arg Lys  
 260 265 270  
 Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285  
 Met Phe Ile Ser Glu Gly Ala Thr Asn Tyr Pro Ser Met Thr Ala Lys  
 290 295 300  
 Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu  
 305 310 315 320  
 Arg Ser Met Met Pro Trp Ile Ser Ala Asn Lys Ile Val Asp Lys Thr  
 325 330 335

Lys Asn

&lt;210&gt; SEQ ID NO 46

&lt;211&gt; LENGTH: 338

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Pseudomonas mendocina

&lt;400&gt; SEQUENCE: 46

Met Lys Val Tyr Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15  
 Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30  
 Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Ile Gly Leu Arg Lys  
 35 40 45  
 Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Thr  
 50 55 60  
 Asp Val Ala Ser Ala Val Ala Ala Ala Asp Leu Val Met Ile Leu Thr  
 65 70 75 80  
 Pro Asp Glu Phe Gln Gly Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
 85 90 95  
 Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
 100 105 110  
 Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125  
 Pro Lys Ala Pro Gly His Thr Val Arg Thr Glu Phe Val Lys Gly Gly  
 130 135 140  
 Gly Ile Pro Asp Leu Ile Ala Val Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160  
 Lys Asn Val Ala Leu Ser Tyr Ala Ser Gly Val Gly Gly Gly Arg Thr  
 165 170 175  
 Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
 195 200 205  
 Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240

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Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
 260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
 290 295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Val Ile Gly Glu Gln Leu  
 305 310 315 320

Arg Ala Met Met Pro Trp Ile Ala Ala Asn Lys Ile Val Asp Lys Thr  
 325 330 335

Lys Asn

&lt;210&gt; SEQ ID NO 47

&lt;211&gt; LENGTH: 336

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus cereus

&lt;400&gt; SEQUENCE: 47

Met Ala Lys Val Tyr Tyr Glu Lys Asp Val Thr Val Asn Val Leu Lys  
 1 5 10 15

Glu Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His  
 20 25 30

Ala Gln Asn Leu Arg Asp Asn Gly Phe Asp Val Val Val Gly Leu Arg  
 35 40 45

Lys Gly Lys Ser Trp Asp Lys Ala Lys Glu Asp Gly Phe Ser Val Tyr  
 50 55 60

Thr Val Ala Glu Ala Ala Lys Gln Ala Asp Val Val Met Ile Leu Leu  
 65 70 75 80

Pro Asp Glu Leu Gln Pro Glu Val Tyr Glu Ala Glu Ile Ala Pro Asn  
 85 90 95

Leu Gln Ala Gly Asn Ser Leu Val Phe Ala His Gly Phe Asn Val His  
 100 105 110

Phe Asp Gln Val Lys Pro Pro Ala Asn Val Asp Val Phe Leu Val Ala  
 115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Ser Glu Gly Gly  
 130 135 140

Ala Val Pro Ala Leu Phe Ala Val Tyr Gln Asp Ala Thr Gly Val Ala  
 145 150 155 160

Thr Glu Lys Ala Leu Ser Tyr Ala Asp Gly Ile Gly Ala Thr Arg Ala  
 165 170 175

Gly Val Leu Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe  
 180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Val Thr Ala Leu Val Lys Ala  
 195 200 205

Gly Phe Glu Thr Leu Val Asp Ala Gly Tyr Gln Pro Glu Leu Ala Tyr  
 210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240

Gly Gly Leu Glu Asn Met Arg Tyr Ser Val Ser Asp Thr Ala Gln Trp  
 245 250 255

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Gly Asp Phe Val Ser Gly Pro Arg Val Val Thr Glu Asp Thr Lys Lys  
 260 265 270

Ala Met Gly Thr Val Leu Ala Glu Ile Gln Asp Gly Thr Phe Ala Arg  
 275 280 285

Gly Trp Ile Ala Glu His Lys Ala Gly Arg Pro Asn Phe His Ala Thr  
 290 295 300

Asn Glu Lys Glu Asn Glu His Glu Ile Glu Val Val Gly Arg Lys Leu  
 305 310 315 320

Arg Glu Met Met Pro Phe Val Gln Pro Arg Val Lys Val Gly Met Lys  
 325 330 335

<210> SEQ ID NO 48  
 <211> LENGTH: 335  
 <212> TYPE: PRT  
 <213> ORGANISM: *Bacillus cereus*

<400> SEQUENCE: 48

Met Lys Thr Tyr Tyr Glu Lys Asp Ala Asn Val Glu Leu Leu Lys Gly  
 1 5 10 15

Lys Thr Val Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30

Gln Asn Leu Arg Asp Ser Gly Val Glu Val Val Val Gly Val Arg Pro  
 35 40 45

Gly Lys Ser Phe Glu Val Ala Lys Thr Asp Gly Phe Glu Val Met Ser  
 50 55 60

Val Ser Glu Ala Val Arg Thr Ala Gln Val Val Gln Met Leu Leu Pro  
 65 70 75 80

Asp Glu Gln Gln Ala His Val Tyr Lys Ala Gly Val Glu Glu Asn Leu  
 85 90 95

Arg Glu Gly Gln Met Leu Leu Phe Ser His Gly Phe Asn Ile His Phe  
 100 105 110

Gly Gln Ile Asn Pro Pro Ser Tyr Val Asp Val Ala Met Val Ala Pro  
 115 120 125

Lys Ser Pro Gly His Leu Val Arg Arg Val Phe Gln Glu Gly Asn Gly  
 130 135 140

Val Pro Ala Leu Val Ala Val His Gln Asp Ala Thr Gly Thr Ala Leu  
 145 150 155 160

His Val Ala Leu Ala Tyr Ala Lys Gly Val Gly Cys Thr Arg Ala Gly  
 165 170 175

Val Ile Glu Thr Thr Phe Gln Glu Glu Thr Glu Thr Asp Leu Phe Gly  
 180 185 190

Glu Gln Thr Val Leu Cys Gly Gly Val Thr Ala Leu Val Lys Ala Gly  
 195 200 205

Phe Glu Thr Leu Thr Glu Gly Gly Tyr Arg Pro Glu Ile Ala Tyr Phe  
 210 215 220

Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu Gly  
 225 230 235 240

Gly Leu Thr Asn Met Arg His Ser Ile Ser Asp Thr Ala Glu Phe Gly  
 245 250 255

Asp Tyr Val Thr Gly Ser Arg Ile Val Thr Asp Glu Thr Lys Lys Glu  
 260 265 270

Met Lys Arg Val Leu Thr Glu Ile Gln Gln Gly Glu Phe Ala Lys Lys

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275                      280                      285  
 Trp Ile Leu Glu Asn Gln Ala Gly Arg Pro Thr Tyr Asn Ala Met Lys  
 290                      295                      300  
 Lys Ala Glu Gln Asn His Gln Leu Glu Lys Val Gly Ala Glu Leu Arg  
 305                      310                      315                      320  
 Glu Met Met Ser Trp Ile Asp Ala Pro Lys Glu Leu Val Lys Lys  
 325                      330                      335

<210> SEQ ID NO 49  
 <211> LENGTH: 22  
 <212> TYPE: DNA  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: primer pBAD-405  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (10)..(11)  
 <223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 49  
 gctcaagcan nkaacctgaa gg 22

<210> SEQ ID NO 50  
 <211> LENGTH: 22  
 <212> TYPE: DNA  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: primer pBAD 427  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (12)..(13)  
 <223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 50  
 ccttcaggtt knntgcttga gc 22

<210> SEQ ID NO 51  
 <211> LENGTH: 21  
 <212> TYPE: DNA  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: primer pBAD435  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (10)..(11)  
 <223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 51  
 gtagacgtgn nkgttgcct g 21

<210> SEQ ID NO 52  
 <211> LENGTH: 21  
 <212> TYPE: DNA  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: primer pBAD456  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (11)..(12)  
 <223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 52  
 caggccaack nncacgtcta c 21

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<210> SEQ ID NO 53  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer pBAD484  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (9)..(10)  
<223> OTHER INFORMATION: n is a, c, g, or t  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (15)..(16)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 53  
  
ctgaagccnn kggcnnkaaa gtgac 25

<210> SEQ ID NO 54  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer pBAD509  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (10)..(11)  
<223> OTHER INFORMATION: n is a, c, g, or t  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (16)..(17)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 54  
  
gtcactttkn ngccknnggc ttcag 25

<210> SEQ ID NO 55  
<211> LENGTH: 22  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Primer pBAD519  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (10)..(11)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 55  
  
gcagccgtdn nkggtgccga ct 22

<210> SEQ ID NO 56  
<211> LENGTH: 22  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Primer pBAD541  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (12)..(13)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 56  
  
agtggcacc knnaacggct gc 22

<210> SEQ ID NO 57  
<211> LENGTH: 22  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence

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<220> FEATURE:  
<223> OTHER INFORMATION: Primer pBAD545  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (11)..(12)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 57  
  
catgatcctg nnkccggacg ag 22  
  
<210> SEQ ID NO 58  
<211> LENGTH: 22  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Primer pBAD567  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (11)..(12)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 58  
  
ctcgtccggk nncaggatca tg 22  
  
<210> SEQ ID NO 59  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Primer pBAD608  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (11)..(12)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 59  
  
caagaagggc nnkactctgg cct 23  
  
<210> SEQ ID NO 60  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Primer 60 pBAD631  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (12)..(13)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 60  
  
aggccagagt knngcccttc ttg 23  
  
<210> SEQ ID NO 61  
<211> LENGTH: 22  
<212> TYPE: DNA  
<213> ORGANISM: artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer pBAD663  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<222> LOCATION: (10)..(11)  
<223> OTHER INFORMATION: n is a, c, g, or t  
  
<400> SEQUENCE: 61  
  
gttgtgectn nkgccgacct cg 22

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<210> SEQ ID NO 62  
 <211> LENGTH: 22  
 <212> TYPE: DNA  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Primer pBAD685  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <222> LOCATION: (12)..(13)  
 <223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 62

cgaggtcggc knnaggcaca ac

22

<210> SEQ ID NO 63  
 <211> LENGTH: 491  
 <212> TYPE: PRT  
 <213> ORGANISM: Escherichia coli

<400> SEQUENCE: 63

Met Ala Asn Tyr Phe Asn Thr Leu Asn Leu Arg Gln Gln Leu Ala Gln  
 1 5 10 15  
 Leu Gly Lys Cys Arg Phe Met Gly Arg Asp Glu Phe Ala Asp Gly Ala  
 20 25 30  
 Ser Tyr Leu Gln Gly Lys Lys Val Val Ile Val Gly Cys Gly Ala Gln  
 35 40 45  
 Gly Leu Asn Gln Gly Leu Asn Met Arg Asp Ser Gly Leu Asp Ile Ser  
 50 55 60  
 Tyr Ala Leu Arg Lys Glu Ala Ile Ala Glu Lys Arg Ala Ser Trp Arg  
 65 70 75 80  
 Lys Ala Thr Glu Asn Gly Phe Lys Val Gly Thr Tyr Glu Glu Leu Ile  
 85 90 95  
 Pro Gln Ala Asp Leu Val Ile Asn Leu Thr Pro Asp Lys Gln His Ser  
 100 105 110  
 Asp Val Val Arg Thr Val Gln Pro Leu Met Lys Asp Gly Ala Ala Leu  
 115 120 125  
 Gly Tyr Ser His Gly Phe Asn Ile Val Glu Val Gly Glu Gln Ile Arg  
 130 135 140  
 Lys Asp Ile Thr Val Val Met Val Ala Pro Lys Cys Pro Gly Thr Glu  
 145 150 155 160  
 Val Arg Glu Glu Tyr Lys Arg Gly Phe Gly Val Pro Thr Leu Ile Ala  
 165 170 175  
 Val His Pro Glu Asn Asp Pro Lys Gly Glu Gly Met Ala Ile Ala Lys  
 180 185 190  
 Ala Trp Ala Ala Ala Thr Gly Gly His Arg Ala Gly Val Leu Glu Ser  
 195 200 205  
 Ser Phe Val Ala Glu Val Lys Ser Asp Leu Met Gly Glu Gln Thr Ile  
 210 215 220  
 Leu Cys Gly Met Leu Gln Ala Gly Ser Leu Leu Cys Phe Asp Lys Leu  
 225 230 235 240  
 Val Glu Glu Gly Thr Asp Pro Ala Tyr Ala Glu Lys Leu Ile Gln Phe  
 245 250 255  
 Gly Trp Glu Thr Ile Thr Glu Ala Leu Lys Gln Gly Gly Ile Thr Leu  
 260 265 270  
 Met Met Asp Arg Leu Ser Asn Pro Ala Lys Leu Arg Ala Tyr Ala Leu  
 275 280 285



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Ser Glu Gln Leu Lys Glu Ile Met Ala Pro Leu Phe Gln Lys His Met  
 290 295 300  
 Asp Asp Ile Ile Ser Gly Glu Phe Ser Ser Gly Met Met Ala Asp Trp  
 305 310 315 320  
 Ala Asn Asp Asp Lys Lys Leu Leu Thr Trp Arg Glu Glu Thr Gly Lys  
 325 330 335  
 Thr Ala Phe Glu Thr Ala Pro Gln Tyr Glu Gly Lys Ile Gly Glu Gln  
 340 345 350  
 Glu Tyr Phe Asp Lys Gly Val Leu Met Ile Ala Met Val Lys Ala Gly  
 355 360 365  
 Val Glu Leu Ala Phe Glu Thr Met Val Asp Ser Gly Ile Ile Glu Glu  
 370 375 380  
 Ser Ala Tyr Tyr Glu Ser Leu His Glu Leu Pro Leu Ile Ala Asn Thr  
 385 390 395 400  
 Ile Ala Arg Lys Arg Leu Tyr Glu Met Asn Val Val Ile Ser Asp Thr  
 405 410 415  
 Ala Glu Tyr Gly Asn Tyr Leu Phe Ser Tyr Ala Cys Val Pro Leu Leu  
 420 425 430  
 Lys Pro Phe Met Ala Glu Leu Gln Pro Gly Asp Leu Gly Lys Ala Ile  
 435 440 445  
 Pro Glu Gly Ala Val Asp Asn Gly Gln Leu Arg Asp Val Asn Glu Ala  
 450 455 460  
 Ile Arg Ser His Ala Ile Glu Gln Val Gly Lys Lys Leu Arg Gly Tyr  
 465 470 475 480  
 Met Thr Asp Met Lys Arg Ile Ala Val Ala Gly  
 485 490

&lt;210&gt; SEQ ID NO 64

&lt;211&gt; LENGTH: 493

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: gamma proteobacterium N4-7

&lt;400&gt; SEQUENCE: 64

Met Ala Asn Tyr Phe Asn Thr Leu Ser Leu Arg Asp Lys Leu Thr Gln  
 1 5 10 15  
 Leu Gly Lys Cys Arg Phe Met Asp Arg Ser Glu Phe Thr Asp Gly Cys  
 20 25 30  
 Asp Phe Ile Lys Asp Trp Asn Ile Val Ile Ile Gly Cys Gly Ala Gln  
 35 40 45  
 Gly Leu Asn Gln Gly Leu Asn Met Arg Asp Ser Gly Leu Asn Ile Ser  
 50 55 60  
 Tyr Ala Leu Arg Ala Gln Ala Ile Ala Glu Lys Arg Gln Ser Phe Val  
 65 70 75 80  
 Trp Ala Ser Glu Asn Gly Phe Thr Val Gly Thr Ala Glu Glu Leu Val  
 85 90 95  
 Pro Ala Ala Asp Leu Val Leu Asn Leu Thr Pro Asp Lys Gln His Thr  
 100 105 110  
 Ala Ala Val Thr Ala Val Met Pro Leu Met Lys Gln Gly Ala Thr Leu  
 115 120 125  
 Ala Tyr Ser His Gly Phe Asn Ile Val Glu Glu Gly Met Gln Ile Arg  
 130 135 140  
 Pro Asp Leu Thr Val Val Met Val Ala Pro Lys Cys Pro Gly Thr Glu

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145	150	155	160
Val Arg Glu Glu Tyr Lys Arg Gly Phe Gly	Val Pro Thr Leu Ile Ala		
165	170	175	
Val His Pro Glu Asn Asp Pro Gln Gly Asn Gly	His Ala Ile Ala Lys		
180	185	190	
Ala Tyr Ala Ser Ala Thr Gly Gly Asp Arg Ala	Gly Val Leu Glu Ser		
195	200	205	
Ser Phe Ile Ala Glu Val Lys Ser Asp Leu Met	Gly Glu Gln Thr Ile		
210	215	220	
Leu Cys Gly Met Leu Gln Thr Gly Ala Val Leu	Gly His Gln Gln Leu		
225	230	235	240
Ile Asn Leu Gly Val Asp Ala Ala Tyr Ala Arg	Lys Leu Ile Gln Tyr		
245	250	255	
Gly Trp Glu Thr Val Thr Glu Gly Leu Lys His	Gly Gly Ile Thr Asn		
260	265	270	
Met Met Asp Arg Leu Ser Asn Pro Ala Lys Ile	Lys Ala Phe Asp Met		
275	280	285	
Ser Glu Glu Leu Lys Val Thr Leu Arg Pro Leu	Phe Glu Lys His Met		
290	295	300	
Asp Asp Ile Ile Glu Gly Glu Phe Ser His Thr	Met Met Ile Asp Trp		
305	310	315	320
Ala Asn Asp Asp Ala Asn Leu Leu Lys Trp Arg	Ala Glu Thr Ala Asp		
325	330	335	
Ser Ser Phe Glu Gln Ala Ala Asp Cys Asp Ile	Glu Ile Thr Glu Gln		
340	345	350	
Glu Phe Tyr Asp Lys Gly Ile Tyr Leu Val Ala	Met Ile Lys Ala Gly		
355	360	365	
Val Glu Leu Ala Phe Glu Thr Met Val Ala Ser	Gly Ile Ile Glu Glu		
370	375	380	
Ser Ala Tyr Tyr Glu Ser Leu His Glu Thr Pro	Leu Ile Ala Asn Cys		
385	390	395	400
Ile Ala Arg Asn Lys Leu Tyr Glu Met Asn Val	Val Ile Ser Asp Thr		
405	410	415	
Ala Glu Tyr Gly Asn Tyr Leu Phe Thr His Ala	Ala Val Pro Leu Leu		
420	425	430	
Gln Ala His Ala Ser Ser Leu Thr Leu Glu Glu	Leu Gly Gly Gly Leu		
435	440	445	
Ala Asp Ser Ser Asn Ala Val Asp Asn Leu Arg	Leu Ile Glu Val Asn		
450	455	460	
Asp Ala Ile Arg Asp His Asp Val Glu Ile Ile	Gly His Glu Leu Arg		
465	470	475	480
Gly Tyr Met Thr Asp Met Lys Arg Ile Val Glu	Ala Gly		
485	490		

&lt;210&gt; SEQ ID NO 65

&lt;211&gt; LENGTH: 490

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Desulfuromonas acetoxidans

&lt;400&gt; SEQUENCE: 65

Met Gly Gln Asn Tyr Phe Asn Thr Leu Ser Met Arg Glu Lys Leu Asp
1 5 10 15

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Glu Leu Gly Thr Cys Arg Phe Met Asp Ala Ser Glu Phe Ala Gly Gly  
 20 25 30  
 Cys Glu Tyr Ala Lys Gly Lys Lys Ile Val Ile Val Gly Cys Gly Ala  
 35 40 45  
 Gln Gly Leu Asn Gln Gly Leu Asn Met Arg Asp Ser Gly Leu Asp Val  
 50 55 60  
 Ser Tyr Thr Leu Arg Lys Glu Ala Ile Ala Glu Lys Arg Gln Ser Tyr  
 65 70 75 80  
 Ile Asn Ala Thr Glu Asn Gly Phe Thr Val Gly Ser Tyr Glu Glu Leu  
 85 90 95  
 Leu Pro Thr Ala Asp Ile Val Met Asn Leu Ala Pro Asp Lys Gln His  
 100 105 110  
 Thr Asp Val Val Asn Thr Val Val Pro Leu Met Lys Gln Gly Ala Thr  
 115 120 125  
 Phe Ser Tyr Ala His Gly Phe Asn Ile Val Glu Glu Gly Thr Ile Ile  
 130 135 140  
 Arg Lys Asp Leu Thr Val Ile Met Val Ala Pro Lys Cys Pro Gly Ser  
 145 150 155 160  
 Glu Val Arg Ala Glu Tyr Gln Arg Gly Phe Gly Val Pro Thr Leu Ile  
 165 170 175  
 Ala Val His Lys Glu Asn Asp Pro Asn Gly Asp Gly Leu Glu Leu Ala  
 180 185 190  
 Lys Ala Leu Cys Ser Ala Gln Gly Gly Asp Arg Ala Gly Val Leu Glu  
 195 200 205  
 Ser Ser Phe Val Ala Glu Val Lys Ser Asp Leu Met Gly Glu Gln Thr  
 210 215 220  
 Ile Leu Cys Gly Met Leu Gln Ala Gly Ala Leu Leu Cys Phe Asp Lys  
 225 230 235 240  
 Met Val Glu Asn Gly Ile Glu Ala Pro Tyr Ala Val Lys Leu Ile Gln  
 245 250 255  
 Tyr Gly Trp Glu Thr Ile Thr Glu Ala Leu Lys His Gly Gly Ile Thr  
 260 265 270  
 Asn Met Met Asp Arg Leu Ser Asn Pro Ala Lys Leu Glu Ala Tyr Glu  
 275 280 285  
 Leu Ala Glu Glu Leu Lys Glu Ile Met Arg Pro Leu Phe Arg Lys His  
 290 295 300  
 Met Asp Asp Ile Ile Thr Gly Val Phe Ser Ser Thr Met Met Glu Asp  
 305 310 315 320  
 Trp Ala Asn Asp Asp Ile Asn Leu Leu Thr Trp Arg Glu Gln Thr Gly  
 325 330 335  
 Gln Thr Ala Phe Glu Lys Thr Glu Ala Ala Gly Glu Ile Ser Glu Gln  
 340 345 350  
 Glu Tyr Phe Asp Lys Ala Ile Leu Met Val Ala Met Val Lys Ala Gly  
 355 360 365  
 Val Glu Leu Ala Phe Glu Ser Met Val Glu Val Gly Ile Glu Pro Glu  
 370 375 380  
 Ser Ala Tyr Tyr Glu Ser Leu His Glu Thr Pro Leu Ile Ala Asn Thr  
 385 390 395 400  
 Ile Ala Arg Lys Lys Leu Tyr Glu Met Asn Arg Val Ile Ser Asp Thr  
 405 410 415  
 Ala Glu Tyr Gly Cys Tyr Leu Phe Ala His Ala Cys Val Pro Leu Leu

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420                425                430
Lys Asp Phe Met Ala Ser Val Thr Thr Glu Val Ile Gly Lys Gly Leu
435                440                445

Asp Asn Val Asp Thr Ser Val Asp Asn Ser Thr Leu Val Arg Val Asn
450                455                460

Ala Asp Ile Arg Ser His Tyr Ile Glu Glu Ile Gly Glu Glu Leu Arg
465                470                475                480

Asp Ala Met Gln Gly Met Lys Ala Ile Val
485                490

<210> SEQ ID NO 66
<211> LENGTH: 581
<212> TYPE: PRT
<213> ORGANISM: Pisum sativum

<400> SEQUENCE: 66

Met Ala Ala Val Thr Ser Ser Cys Ser Thr Ala Ile Ser Ala Ser Ser
1                5                10                15

Lys Thr Leu Ala Lys Pro Val Ala Ala Ser Phe Ala Pro Thr Asn Leu
20                25                30

Ser Phe Ser Lys Leu Ser Pro Gln Ser Ile Arg Ala Arg Arg Ser Ile
35                40                45

Thr Val Gly Ser Ala Leu Gly Ala Thr Lys Val Ser Ala Pro Pro Ala
50                55                60

Thr His Pro Val Ser Leu Asp Phe Glu Thr Ser Val Phe Lys Lys Glu
65                70                75                80

Arg Val Asn Leu Ala Gly His Glu Glu Tyr Ile Val Arg Gly Gly Arg
85                90                95

Asp Leu Phe His Leu Leu Pro Asp Ala Phe Lys Gly Ile Lys Gln Ile
100               105               110

Gly Val Ile Gly Trp Gly Ser Gln Gly Pro Ala Gln Ala Gln Asn Leu
115               120               125

Arg Asp Ser Leu Val Glu Ala Lys Ser Asp Ile Val Val Lys Val Gly
130               135               140

Leu Arg Lys Gly Ser Ser Ser Phe Asn Glu Ala Arg Glu Ala Gly Phe
145               150               155               160

Ser Glu Glu Lys Gly Thr Leu Gly Asp Ile Trp Glu Thr Ile Ser Gly
165               170               175

Ser Asp Leu Val Leu Leu Leu Ile Ser Asp Ser Ala Gln Ala Asp Asn
180               185               190

Tyr Glu Lys Ile Phe Ser His Leu Lys Pro Asn Ser Ile Leu Gly Leu
195               200               205

Ser His Gly Phe Leu Leu Gly His Leu Gln Ser Ile Gly Leu Asp Phe
210               215               220

Pro Lys Asn Phe Ser Val Ile Ala Val Cys Pro Lys Gly Met Gly Pro
225               230               235               240

Ser Val Arg Arg Leu Tyr Val Gln Gly Lys Glu Ile Asn Gly Ala Gly
245               250               255

Ile Asn Ser Ser Phe Gly Val His Gln Asp Val Asp Gly Arg Ala Thr
260               265               270

Asn Val Ala Leu Gly Trp Ser Val Ala Leu Gly Ser Pro Phe Thr Phe
275               280               285

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Ala Thr Thr Leu Glu Gln Glu Tyr Lys Ser Asp Ile Phe Gly Glu Arg  
 290 295 300

Gly Ile Leu Leu Gly Ala Val His Gly Ile Val Glu Ser Leu Phe Arg  
 305 310 315 320

Arg Tyr Thr Glu Asn Gly Met Ser Glu Asp Leu Ala Tyr Lys Asn Thr  
 325 330 335

Val Glu Ser Ile Thr Gly Val Ile Ser Lys Thr Ile Ser Thr Gln Gly  
 340 345 350

Met Leu Ala Val Tyr Asn Ala Leu Ser Glu Asp Gly Lys Lys Glu Phe  
 355 360 365

Glu Lys Ala Tyr Ser Ala Ser Phe Tyr Pro Cys Met Glu Ile Leu Tyr  
 370 375 380

Glu Cys Tyr Glu Asp Val Ala Ser Gly Ser Glu Ile Arg Ser Val Val  
 385 390 395 400

Leu Ala Gly Arg Arg Phe Tyr Glu Lys Glu Gly Leu Pro Ala Phe Pro  
 405 410 415

Met Gly Lys Ile Asp Gln Thr Arg Met Trp Lys Val Gly Glu Arg Val  
 420 425 430

Arg Ser Thr Arg Pro Ala Gly Asp Leu Gly Pro Leu Tyr Pro Phe Thr  
 435 440 445

Ala Gly Val Phe Val Ala Met Met Met Ala Gln Ile Glu Val Leu Arg  
 450 455 460

Lys Lys Gly His Ser Tyr Ser Glu Ile Ile Asn Glu Ser Val Ile Glu  
 465 470 475 480

Ser Val Asp Ser Leu Asn Pro Phe Met His Ala Arg Gly Val Ser Phe  
 485 490 495

Met Val Asp Asn Cys Ser Thr Thr Ala Arg Leu Gly Ser Arg Lys Trp  
 500 505 510

Ala Pro Arg Phe Asp Tyr Ile Leu Thr Gln Gln Ala Leu Val Ala Val  
 515 520 525

Asp Ser Gly Ala Pro Ile Asn Gln Asp Leu Ile Ser Asn Phe Val Ser  
 530 535 540

Asp Pro Val His Gly Ala Ile Gln Val Cys Ala Glu Leu Arg Pro Thr  
 545 550 555 560

Leu Asp Ile Ser Val Pro Ala Ala Ala Asp Phe Val Arg Pro Glu Leu  
 565 570 575

Arg Gln Cys Ser Asn  
 580

<210> SEQ ID NO 67  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: mutant KARI 3361G8

<400> SEQUENCE: 67

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30

Leu Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys  
 35 40 45

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Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr  
 50 55 60  
 Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Ile  
 65 70 75 80  
 Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn  
 85 90 95  
 Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His  
 100 105 110  
 Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala  
 115 120 125  
 Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly  
 130 135 140  
 Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala  
 145 150 155 160  
 Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr  
 165 170 175  
 Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe  
 180 185 190  
 Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala  
 195 200 205  
 Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr  
 210 215 220  
 Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu  
 225 230 235 240  
 Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr  
 245 250 255  
 Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln  
 260 265 270  
 Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys  
 275 280 285  
 Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys  
 290 295 300  
 Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu  
 305 310 315 320  
 Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala  
 325 330 335

Lys Asn

<210> SEQ ID NO 68  
 <211> LENGTH: 338  
 <212> TYPE: PRT  
 <213> ORGANISM: artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: KARI mutant 2H10

&lt;400&gt; SEQUENCE: 68

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly  
 1 5 10 15  
 Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala  
 20 25 30  
 Leu Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys  
 35 40 45  
 Gly Ala Ala Asp Ile Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr

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50              55              60
Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Ile
65              70              75              80
Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn
85              90              95
Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His
100             105             110
Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala
115             120             125
Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly
130             135             140
Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Val Ser Gly Asn Ala
145             150             155             160
Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr
165             170             175
Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe
180             185             190
Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala
195             200             205
Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
210             215             220
Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225             230             235             240
Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr
245             250             255
Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln
260             265             270
Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys
275             280             285
Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys
290             295             300
Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu
305             310             315             320
Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala
325             330             335
Lys Asn

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<210> SEQ ID NO 69
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: KARI mutant 1D2

<400> SEQUENCE: 69

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Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly
1              5              10              15
Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala
20             25             30
Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys
35             40             45
Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr
50             55             60

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Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Ile
65          70          75          80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn
85          90          95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His
100         105         110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala
115         120         125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly
130         135         140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Val Ser Gly Asn Ala
145         150         155         160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr
165         170         175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe
180         185         190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala
195         200         205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
210         215         220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225         230         235         240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr
245         250         255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln
260         265         270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys
275         280         285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys
290         295         300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu
305         310         315         320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala
325         330         335

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Lys Asn

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<210> SEQ ID NO 70
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: KARI mutant 3F12

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&lt;400&gt; SEQUENCE: 70

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Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly
1          5          10         15

Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala
20         25         30

Leu Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Tyr Lys
35         40         45

Gly Ala Ala Asp Ala Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr
50         55         60

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Asp	Val	Ala	Ala	Ala	Val	Ala	Gly	Ala	Asp	Leu	Val	Met	Ile	Leu	Ile
65					70					75					80
Pro	Asp	Glu	Phe	Gln	Ser	Gln	Leu	Tyr	Lys	Asn	Glu	Ile	Glu	Pro	Asn
85					90					95					
Ile	Lys	Lys	Gly	Ala	Thr	Leu	Ala	Phe	Ser	His	Gly	Phe	Ala	Ile	His
100					105					110					
Tyr	Asn	Gln	Val	Val	Pro	Arg	Ala	Asp	Leu	Asp	Val	Ile	Met	Ile	Ala
115					120					125					
Pro	Lys	Ala	Pro	Gly	His	Thr	Val	Arg	Ser	Glu	Phe	Val	Lys	Gly	Gly
130					135					140					
Gly	Ile	Pro	Asp	Leu	Ile	Ala	Ile	Tyr	Gln	Asp	Val	Ser	Gly	Asn	Ala
145					150					155					160
Lys	Asn	Val	Ala	Leu	Ser	Tyr	Ala	Ala	Gly	Val	Gly	Gly	Gly	Arg	Thr
165					170					175					
Gly	Ile	Ile	Glu	Thr	Thr	Phe	Lys	Asp	Glu	Thr	Glu	Thr	Asp	Leu	Phe
180					185					190					
Gly	Glu	Gln	Ala	Val	Leu	Cys	Gly	Gly	Thr	Val	Glu	Leu	Val	Lys	Ala
195					200					205					
Gly	Phe	Glu	Thr	Leu	Val	Glu	Ala	Gly	Tyr	Ala	Pro	Glu	Met	Ala	Tyr
210					215					220					
Phe	Glu	Cys	Leu	His	Glu	Leu	Lys	Leu	Ile	Val	Asp	Leu	Met	Tyr	Glu
225					230					235					240
Gly	Gly	Ile	Ala	Asn	Met	Asn	Tyr	Ser	Ile	Ser	Asn	Asn	Ala	Glu	Tyr
245					250					255					
Gly	Glu	Tyr	Val	Thr	Gly	Pro	Glu	Val	Ile	Asn	Ala	Glu	Ser	Arg	Gln
260					265					270					
Ala	Met	Arg	Asn	Ala	Leu	Lys	Arg	Ile	Gln	Asp	Gly	Glu	Tyr	Ala	Lys
275					280					285					
Met	Phe	Ile	Ser	Glu	Gly	Ala	Thr	Gly	Tyr	Pro	Ser	Met	Thr	Ala	Lys
290					295					300					
Arg	Arg	Asn	Asn	Ala	Ala	His	Gly	Ile	Glu	Ile	Ile	Gly	Glu	Gln	Leu
305					310					315					320
Arg	Ser	Met	Met	Pro	Trp	Ile	Gly	Ala	Asn	Lys	Ile	Val	Asp	Lys	Ala
325					330					335					

Lys Asn

What is claimed is:

1. A mutant ketol-acid reductoisomerase enzyme comprising the amino acid sequence as set forth in SEQ ID NO: 29.

2. A nucleic acid molecule encoding the mutant ketol-acid reductoisomerase enzyme of claim 1.

3. A nucleic acid molecule encoding a mutant ketol-acid reductoisomerase enzyme have the nucleic acid sequence as set forth in SEQ ID NO:23.

4. A mutant ketol-acid reductoisomerase enzyme encoded by the nucleic acid molecule of claim 3.

5. A recombinant cell comprising the mutant ketol-acid reductoisomerase enzyme of claim 1.

6. A mutant ketol-acid reductoisomerase enzyme as set forth in SEQ ID NO:17 comprising at least one mutation at a residue selected from the group consisting of 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, 165, and 170.

7. A mutant ketol-acid reductoisomerase enzyme according to claim 6 wherein:

a) the residue at position 47 has an amino acid substitution selected from the group consisting of A, C, D, F, G, I, L, N, P, and Y;

b) the residue at position 50 has an amino acid substitution selected from the group consisting of A, C, D, E, F, G, M, N, V, W;

c) the residue at position 52 has an amino acid substitution selected from the group consisting of A, C, D, G, H, N, S;

d) the residue at position 53 has an amino acid substitution selected from the group consisting of A, H, I, W;

e) the residue at position 156 has an amino acid substitution of V;

f) the residue at position 165 has an amino acid substitution of M;

- g) the residue at position 61 has an amino acid substitution of F;
- h) the residue at position 170 has an amino acid substitution of A;
- i) the residue at position 24 has an amino acid substitution of F;
- j) the residue at position 33 has an amino acid substitution of L;
- k) the residue at position 80 has an amino acid substitution of I; and
- l) the residue at position 115 has an amino acid substitution of L.

**8.** A nucleic acid molecule encoding the mutant ketol-acid reductoisomerase enzyme of claim 6.

**9.** A method for the evolution of a NADPH binding ketol-acid reductoisomerase enzyme to an NADH using form comprising:

- a) providing a ketol-acid reductoisomerase enzyme which uses NADPH having a specific native amino acid sequence;
- b) identifying the cofactor switching residues in the enzyme of a) based on the amino acid sequence of the *Pseudomonas fluorescens* ketol-acid reductoisomerase enzyme as set for the in SEQ ID NO:17 wherein the cofactor switching residues are at positions selected from the group consisting of; 24, 33, 47, 50, 52, 53, 61, 80, 115, 156, 165, and 170.
- c) creating mutations in at least one of the cofactor switching residues of b) to create a mutant enzyme wherein said mutant enzyme binds NADH.

**10.** The method of claim 9 wherein:

- a) the residue at position 47 has an amino acid substitution selected from the group consisting of A, C, D, F, G, I, L, N, P, and Y;
- b) the residue at position 50 has an amino acid substitution selected from the group consisting of A, C, D, E, F, G, M, N, V, W;
- c) the residue at position 52 has an amino acid substitution selected from the group consisting of A, C, D, G, H, N, S;
- d) the residue at position 53 has an amino acid substitution selected from the group consisting of A, H, I, W;
- e) the residue at position 156 has an amino acid substitution of V;
- f) the residue at position 165 has an amino acid substitution of M;
- g) the residue at position 61 has an amino acid substitution of F;
- h) the residue at position 170 has an amino acid substitution of A;
- i) the residue at position 24 has an amino acid substitution of F;

- j) the residue at position 33 has an amino acid substitution of L;
- k) the residue at position 80 has an amino acid substitution of I; and
- l) the residue at position 115 has an amino acid substitution of L.

**11.** The method of claim 9 wherein the ketol-acid reductoisomerase enzyme has the amino acid sequence as set forth in SEQ ID NO: 29.

**12.** A method for the production of isobutanol comprising:

- a) providing a recombinant microbial host cell comprising the following genetic constructs:
  - i) at least one genetic construct encoding an acetolactate synthase enzyme for the conversion of pyruvate to acetolactate;
  - ii) at least one genetic construct encoding a ketol-acid reductoisomerase enzyme of either of claims 1 or 6;
  - iii) at least one genetic construct encoding an acetohydroxy acid dehydratase for the conversion of 2,3-dihydroxyisovalerate to  $\alpha$ -ketoisovalerate, (pathway step c);
  - iv) at least one genetic construct encoding a branched-chain keto acid decarboxylase, of the conversion of  $\alpha$ -ketoisovalerate to isobutyraldehyde, (pathway step d);
  - v) at least one genetic construct encoding a branched-chain alcohol dehydrogenase for the conversion of isobutyraldehyde to isobutanol (pathway step e); and
- b) growing the host cell of (a) under conditions where iso-butanol is produced.

**13.** A method for the evolution and identification of a NADPH binding ketol-acid reductoisomerase enzyme to an NADH using form comprising:

- a) providing a ketol-acid reductoisomerase enzyme which uses NADPH having a specific native amino acid sequence;
- b) identifying the amino acid residues in the native amino acid sequence whose side chains are in close proximity to the adenosyl 2'-phosphate of NADPH as mutagenesis targets;
- c) creating a library of mutant ketol-acid reductoisomerase enzymes from the class I ketol-acid reductoisomerase enzyme of step (a), having at least one mutation in at least one of the mutagenesis target sites of step (b); and
- d) screening the library of mutant ketol-acid reductoisomerase enzymes of step (c) to identify NADH binding mutant ketol-acid reductoisomerase enzyme.

**14.** A mutant ketol-acid reductoisomerase enzyme having the amino acid sequence selected from the group consisting of SEQ ID NO: 19, 24, 25, 26, 27, 28, 67, 68, 69, and 70.

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