

12956_192_SEQLIST

SEQUENCE LISTING

<110> Genomatica, Inc.

<120> EUKARYOTIC ORGANISMS AND METHODS FOR INCREASING THE
AVAILABILITY OF CYTOSOLIC ACETYL-COA, AND FOR
PRODUCING 1,3-BUTANEDIOL

<130> 12956-192-228

<140> TBA

<141> 2012-09-07

<150> 61/532,492

<151> 2011-09-08

<150> 61/541,951

<151> 2011-09-30

<150> 61/558,959

<151> 2011-11-11

<150> 61/649,039

<151> 2012-05-18

<150> 61/655,355

<151> 2012-06-04

<160> 8

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 59

<212> DNA

<213> Artificial Sequence

<220>

<223> lacZ alpha-RI primer for PCR amplification

<400> 1

gacgaattcg ctagcaagag gagaagtcga catgtccaat tcaactggccg tcgttttac 59

<210> 2

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> lacZ alpha-3-prime BB primer for PCR amplification

<400> 2

gaccctagga agctttctag agtcgaccta tgcggcatca gagcaga 47

<210> 3

<211> 474

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutated LpdA from E. coli K-12 MG1655

<400> 3

Met	Ser	Thr	Glu	Ile	Lys	Thr	Gln	Val	Val	Val	Leu	Gly	Ala	Gly	Pro
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Ala	Gly	Tyr	Ser	Ala	Ala	Phe	Arg	Cys	Ala	Asp	Leu	Gly	Leu	Glu	Thr
			20					25				30			

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Val Ile Val Glu Arg Tyr Asn Thr Leu Gly Gly Val Cys Leu Asn Val
 35 40 45
 Gly Cys Ile Pro Ser Lys Ala Leu Leu His Val Ala Lys Val Ile Glu
 50 55 60
 Glu Ala Lys Ala Leu Ala Glu His Gly Ile Val Phe Gly Glu Pro Lys
 65 70 75 80
 Thr Asp Ile Asp Lys Ile Arg Thr Trp Lys Glu Lys Val Ile Asn Gln
 85 90 95
 Leu Thr Gly Gly Leu Ala Gly Met Ala Lys Gly Arg Lys Val Lys Val
 100 105 110
 Val Asn Gly Leu Gly Lys Phe Thr Gly Ala Asn Thr Leu Glu Val Glu
 115 120 125
 Gly Glu Asn Gly Lys Thr Val Ile Asn Phe Asp Asn Ala Ile Ile Ala
 130 135 140
 Ala Gly Ser Arg Pro Ile Gln Leu Pro Phe Ile Pro His Glu Asp Pro
 145 150 155 160
 Arg Ile Trp Asp Ser Thr Asp Ala Leu Glu Leu Lys Glu Val Pro Glu
 165 170 175
 Arg Leu Leu Val Met Gly Gly Gly Ile Gly Leu Glu Met Gly Thr
 180 185 190
 Val Tyr His Ala Leu Gly Ser Gln Ile Asp Val Val Val Arg Lys His
 195 200 205
 Gln Val Ile Arg Ala Ala Asp Lys Asp Ile Val Lys Val Phe Thr Lys
 210 215 220
 Arg Ile Ser Lys Lys Phe Asn Leu Met Leu Glu Thr Lys Val Thr Ala
 225 230 235 240
 Val Glu Ala Lys Glu Asp Gly Ile Tyr Val Thr Met Glu Gly Lys Lys
 245 250 255
 Ala Pro Ala Glu Pro Gln Arg Tyr Asp Ala Val Leu Val Ala Ile Gly
 260 265 270
 Arg Val Pro Asn Gly Lys Asn Leu Asp Ala Gly Lys Ala Gly Val Glu
 275 280 285
 Val Asp Asp Arg Gly Phe Ile Arg Val Asp Lys Gln Leu Arg Thr Asn
 290 295 300
 Val Pro His Ile Phe Ala Ile Gly Asp Ile Val Gly Gln Pro Met Leu
 305 310 315 320
 Ala His Lys Gly Val His Glu Gly His Val Ala Ala Glu Val Ile Ala
 325 330 335
 Gly Lys Lys His Tyr Phe Asp Pro Lys Val Ile Pro Ser Ile Ala Tyr
 340 345 350
 Thr Glu Pro Glu Val Ala Trp Val Gly Leu Thr Glu Lys Glu Ala Lys
 355 360 365
 Glu Lys Gly Ile Ser Tyr Glu Thr Ala Thr Phe Pro Trp Ala Ala Ser
 370 375 380
 Gly Arg Ala Ile Ala Ser Asp Cys Ala Asp Gly Met Thr Lys Leu Ile
 385 390 395 400
 Phe Asp Lys Glu Ser His Arg Val Ile Gly Gly Ala Ile Val Gly Thr
 405 410 415
 Asn Gly Gly Glu Leu Leu Gly Glu Ile Gly Leu Ala Ile Glu Met Gly
 420 425 430
 Cys Asp Ala Glu Asp Ile Ala Leu Thr Ile His Ala His Pro Thr Leu
 435 440 445
 His Glu Ser Val Gly Leu Ala Ala Glu Val Phe Glu Gly Ser Ile Thr
 450 455 460
 Asp Leu Pro Asn Pro Lys Ala Lys Lys Lys
 465 470

<210> 4

<211> 474

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutated LpdA from E. coli K-12 MG1655

<400> 4

Met Ser Thr Glu Ile Lys Thr Gln Val Val Val Leu Gly Ala Gly Pro

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1	Ala	Gly	Tyr	Ser	5	Ala	Ala	Phe	Arg	Cys	10	Ala	Asp	Leu	Gly	Leu	15	Glu	Thr
				20						25						30			
Val	Ile	Val	Glu	Arg	Tyr	Asn	Thr	Leu	Gly	Gly	Val	Cys	Leu	Asn	Val				
		35					40						45						
Gly	Cys	Ile	Pro	Ser	Lys	Ala	Leu	Leu	His	Val	Ala	Lys	Val	Ile	Glu				
	50					55					60								
Glu	Ala	Lys	Ala	Leu	Ala	Glu	His	Gly	Ile	Val	Phe	Gly	Glu	Pro	Lys				
65					70					75						80			
Thr	Asp	Ile	Asp	Lys	Ile	Arg	Thr	Trp	Lys	Glu	Lys	Val	Ile	Asn	Gln				
			85						90					95					
Leu	Thr	Gly	Gly	Leu	Ala	Gly	Met	Ala	Lys	Gly	Arg	Lys	Val	Lys	Val				
			100					105						110					
Val	Asn	Gly	Leu	Gly	Lys	Phe	Thr	Gly	Ala	Asn	Thr	Leu	Glu	Val	Glu				
		115					120						125						
Gly	Glu	Asn	Gly	Lys	Thr	Val	Ile	Asn	Phe	Asp	Asn	Ala	Ile	Ile	Ala				
	130					135					140								
Ala	Gly	Ser	Arg	Pro	Ile	Gln	Leu	Pro	Phe	Ile	Pro	His	Glu	Asp	Pro				
145					150					155					160				
Arg	Ile	Trp	Asp	Ser	Thr	Asp	Ala	Leu	Glu	Leu	Lys	Glu	Val	Pro	Glu				
			165						170						175				
Arg	Leu	Leu	Val	Met	Gly	Gly	Gly	Ile	Ile	Ala	Leu	Glu	Met	Ala	Thr				
			180					185						190					
Val	Tyr	His	Ala	Leu	Gly	Ser	Gln	Ile	Asp	Val	Val	Val	Arg	Lys	His				
		195					200						205						
Gln	Val	Ile	Arg	Ala	Ala	Asp	Lys	Asp	Ile	Val	Lys	Val	Phe	Thr	Lys				
	210					215					220								
Arg	Ile	Ser	Lys	Lys	Phe	Asn	Leu	Met	Leu	Glu	Thr	Lys	Val	Thr	Ala				
225					230					235					240				
Val	Glu	Ala	Lys	Glu	Asp	Gly	Ile	Tyr	Val	Thr	Met	Glu	Gly	Lys	Lys				
			245						250					255					
Ala	Pro	Ala	Glu	Pro	Gln	Arg	Tyr	Asp	Ala	Val	Leu	Val	Ala	Ile	Gly				
			260					265						270					
Arg	Val	Pro	Asn	Gly	Lys	Asn	Leu	Asp	Ala	Gly	Lys	Ala	Gly	Val	Glu				
		275					280							285					
Val	Asp	Asp	Arg	Gly	Phe	Ile	Arg	Val	Asp	Lys	Gln	Leu	Arg	Thr	Asn				
	290					295					300								
Val	Pro	His	Ile	Phe	Ala	Ile	Gly	Asp	Ile	Val	Gly	Gln	Pro	Met	Leu				
305					310					315					320				
Ala	His	Lys	Gly	Val	His	Glu	Gly	His	Val	Ala	Ala	Glu	Val	Ile	Ala				
			325						330					335					
Gly	Lys	Lys	His	Tyr	Phe	Asp	Pro	Lys	Val	Ile	Pro	Ser	Ile	Ala	Tyr				
			340					345					350						
Thr	Glu	Pro	Glu	Val	Ala	Trp	Val	Gly	Leu	Thr	Glu	Lys	Glu	Ala	Lys				
		355					360						365						
Glu	Lys	Gly	Ile	Ser	Tyr	Glu	Thr	Ala	Thr	Phe	Pro	Trp	Ala	Ala	Ser				
	370					375					380								
Gly	Arg	Ala	Ile	Ala	Ser	Asp	Cys	Ala	Asp	Gly	Met	Thr	Lys	Leu	Ile				
385					390					395					400				
Phe	Asp	Lys	Glu	Ser	His	Arg	Val	Ile	Gly	Gly	Ala	Ile	Val	Gly	Thr				
			405					410						415					
Asn	Gly	Gly	Glu	Leu	Leu	Gly	Glu	Ile	Gly	Leu	Ala	Ile	Glu	Met	Gly				
			420					425					430						
Cys	Asp	Ala	Glu	Asp	Ile	Ala	Leu	Thr	Ile	His	Ala	His	Pro	Thr	Leu				
		435					440						445						
His	Glu	Ser	Val	Gly	Leu	Ala	Ala	Glu	Val	Phe	Glu	Gly	Ser	Ile	Thr				
	450					455					460								
Asp	Leu	Pro	Asn	Pro	Lys	Ala	Lys	Lys	Lys										
465					470														

<210> 5

<211> 364

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Candida bodinii enzyme

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<400> 5

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Met Lys Ile Val Leu Val Leu Tyr Asp Ala Gly Lys His Ala Ala Asp
 1      5      10      15
Glu Glu Lys Leu Tyr Gly Cys Thr Glu Asn Lys Leu Gly Ile Ala Asn
 20      25      30
Trp Leu Lys Asp Gln Gly His Glu Leu Ile Thr Thr Ser Asp Lys Glu
 35      40      45
Gly Glu Thr Ser Glu Leu Asp Lys His Ile Pro Asp Ala Asp Ile Ile
 50      55      60
Ile Thr Thr Pro Phe His Pro Ala Tyr Ile Thr Lys Glu Arg Leu Asp
 65      70      75      80
Lys Ala Lys Asn Leu Lys Leu Val Val Val Ala Gly Val Gly Ser Asp
 85      90      95
His Ile Asp Leu Asp Tyr Ile Asn Gln Thr Gly Lys Lys Ile Ser Val
100      105      110
Leu Glu Val Thr Gly Ser Asn Val Val Ser Val Ala Glu His Val Val
115      120      125
Met Thr Met Leu Val Leu Val Arg Asn Phe Val Pro Ala His Glu Gln
130      135      140
Ile Ile Asn His Asp Trp Glu Val Ala Ala Ile Ala Lys Asp Ala Tyr
145      150      155      160
Asp Ile Glu Gly Lys Thr Ile Ala Thr Ile Gly Ala Gly Arg Ile Gly
165      170      175
Tyr Arg Val Leu Glu Arg Leu Leu Pro Phe Asn Pro Lys Glu Leu Leu
180      185      190
Tyr Tyr Gln Arg Gln Ala Leu Pro Lys Glu Ala Glu Glu Lys Val Gly
195      200      205
Ala Arg Arg Val Glu Asn Ile Glu Glu Leu Val Ala Gln Ala Asp Ile
210      215      220
Val Thr Val Asn Ala Pro Leu His Ala Gly Thr Lys Gly Leu Ile Asn
225      230      235      240
Lys Glu Leu Leu Ser Lys Phe Lys Lys Gly Ala Trp Leu Val Asn Thr
245      250      255
Ala Arg Gly Ala Ile Cys Val Ala Glu Asp Val Ala Ala Ala Leu Glu
260      265      270
Ser Gly Gln Leu Arg Gly Tyr Gly Asp Val Trp Phe Pro Gln Pro
275      280      285
Ala Pro Lys Asp His Pro Trp Arg Asp Met Arg Asn Lys Tyr Gly Ala
290      295      300
Gly Asn Ala Met Thr Pro His Tyr Ser Gly Thr Thr Leu Asp Ala Gln
305      310      315      320
Thr Arg Tyr Ala Glu Gly Thr Lys Asn Ile Leu Glu Ser Phe Phe Thr
325      330      335
Gly Lys Phe Asp Tyr Arg Pro Gln Asp Ile Ile Leu Leu Asn Gly Glu
340      345      350
Tyr Val Thr Lys Ala Tyr Gly Lys His Asp Lys Lys
355      360

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<210> 6

<211> 364

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant Candida bodinii enzyme

<400> 6

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

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65      70      75      80
Lys Ala Lys Asn Leu Lys Leu Val Val Val Ala Gly Val Gly Ser Asp
      85      90      95
His Ile Asp Leu Asp Tyr Ile Asn Gln Thr Gly Lys Lys Ile Ser Val
      100      105      110
Leu Glu Val Thr Gly Ser Asn Val Val Ser Val Ala Glu His Val Val
      115      120      125
Met Thr Met Leu Val Leu Val Arg Asn Phe Val Pro Ala His Glu Gln
      130      135      140
Ile Ile Asn His Asp Trp Glu Val Ala Ala Ile Ala Lys Asp Ala Tyr
145      150      155      160
Asp Ile Glu Gly Lys Thr Ile Ala Thr Ile Gly Ala Gly Arg Ile Gly
      165      170      175
Tyr Arg Val Leu Glu Arg Leu Leu Pro Phe Asn Pro Lys Glu Leu Leu
      180      185      190
Tyr Tyr Ser Pro Gln Ala Leu Pro Lys Glu Ala Glu Glu Lys Val Gly
      195      200      205
Ala Arg Arg Val Glu Asn Ile Glu Glu Leu Val Ala Gln Ala Asp Ile
210      215      220
Val Thr Val Asn Ala Pro Leu His Ala Gly Thr Lys Gly Leu Ile Asn
225      230      235      240
Lys Glu Leu Leu Ser Lys Phe Lys Lys Gly Ala Trp Leu Val Asn Thr
      245      250      255
Ala Arg Gly Ala Ile Cys Val Ala Glu Asp Val Ala Ala Ala Leu Glu
      260      265      270
Ser Gly Gln Leu Arg Gly Tyr Gly Asp Val Trp Phe Pro Gln Pro
      275      280      285
Ala Pro Lys Asp His Pro Trp Arg Asp Met Arg Asn Lys Tyr Gly Ala
290      295      300
Gly Asn Ala Met Thr Pro His Tyr Ser Gly Thr Thr Leu Asp Ala Gln
305      310      315      320
Thr Arg Tyr Ala Glu Gly Thr Lys Asn Ile Leu Glu Ser Phe Phe Thr
      325      330      335
Gly Lys Phe Asp Tyr Arg Pro Gln Asp Ile Ile Leu Leu Asn Gly Glu
      340      345      350
Tyr Val Thr Lys Ala Tyr Gly Lys His Asp Lys Lys
      355      360

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<210> 7

<211> 376

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant *Saccharomyces cerevisiae* enzyme

<400> 7

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

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12956_192_SEQLIST

Glu Tyr Asp Leu Glu Asp Lys Ile Ile Ser Thr Val Gly Ala Gly Arg
 165 170 175
 Ile Gly Tyr Arg Val Leu Glu Arg Leu Val Ala Phe Asn Pro Lys Lys
 180 185 190
 Leu Leu Tyr Tyr Ala Arg Gln Glu Leu Pro Ala Glu Ala Ile Asn Arg
 195 200 205
 Leu Asn Glu Ala Ser Lys Leu Phe Asn Gly Arg Gly Asp Ile Val Gln
 210 215 220
 Arg Val Glu Lys Leu Glu Asp Met Val Ala Gln Ser Asp Val Val Thr
 225 230 235 240
 Ile Asn Cys Pro Leu His Lys Asp Ser Arg Gly Leu Phe Asn Lys Lys
 245 250 255
 Leu Ile Ser His Met Lys Asp Gly Ala Tyr Leu Val Asn Thr Ala Arg
 260 265 270
 Gly Ala Ile Cys Val Ala Glu Asp Val Ala Glu Ala Val Lys Ser Gly
 275 280 285
 Lys Leu Ala Gly Tyr Gly Gly Asp Val Trp Asp Lys Gln Pro Ala Pro
 290 295 300
 Lys Asp His Pro Trp Arg Thr Met Asp Asn Lys Asp His Val Gly Asn
 305 310 315 320
 Ala Met Thr Val His Ile Ser Gly Thr Ser Leu Asp Ala Gln Lys Arg
 325 330 335
 Tyr Ala Gln Gly Val Lys Asn Ile Leu Asn Ser Tyr Phe Ser Lys Lys
 340 345 350
 Phe Asp Tyr Arg Pro Gln Asp Ile Val Gln Asn Gly Ser Tyr Ala
 355 360 365
 Thr Arg Ala Tyr Gly Gln Lys Lys
 370 375

<210> 8

<211> 20

<212> PRT

<213> Euglena gracilis

<220>

<223> first 20 amino acids starting from the N-terminus of a
KDC from Euglena gracilis

<400> 8

Met Thr Tyr Lys Ala Pro Val Lys Asp Val Lys Phe Leu Leu Asp Lys
 1 5 10 15
 Val Phe Lys Val
 20