

OPA15135_Sequence Listing

<110> CJ CheilJedang Corporation
 <120> Microorganism producing O-acetyl-homoserine and method for
 producing O-Acetyl-homoserine using the same
 <130> OPA15135
 <150> KR10-2014-0068613
 <151> 2014-06-05
 <160> 53
 <170> KopatentIn 2.0
 <210> 1
 <211> 418
 <212> PRT
 <213> E.coli yjeH
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 Leu Ser Thr Ser Leu Leu Gly Thr Gly Val Phe Ala Val Pro Ala Leu
 20 25 30
 Ala Ala Leu Val Ala Gly Asn Asn Ser Leu Trp Ala Trp Pro Val Leu
 35 40 45
 Ile Ile Leu Val Phe Pro Ile Ala Ile Val Phe Ala Ile Leu Gly Arg
 50 55 60
 His Tyr Pro Ser Ala Gly Gly Val Ala His Phe Val Gly Met Ala Phe
 65 70 75 80
 Gly Ser Arg Leu Glu Arg Val Thr Gly Trp Leu Phe Leu Ser Val Ile
 85 90 95
 Pro Val Gly Leu Pro Ala Ala Leu Gln Ile Ala Ala Gly Phe Gly Gln
 100 105 110
 Ala Met Phe Gly Trp His Ser Trp Gln Leu Leu Leu Ala Glu Leu Gly
 115 120 125
 Thr Leu Ala Leu Val Trp Tyr Ile Gly Thr Arg Gly Ala Ser Ser Ser
 130 135 140
 Ala Asn Leu Gln Thr Val Ile Ala Gly Leu Ile Val Ala Leu Ile Val
 145 150 155 160
 Ala Ile Trp Trp Ala Gly Asp Ile Lys Pro Ala Asn Ile Pro Phe Pro
 165 170 175
 Ala Pro Gly Asn Ile Glu Leu Thr Gly Leu Phe Ala Ala Leu Ser Val
 180 185 190
 Met Phe Trp Cys Phe Val Gly Leu Glu Ala Phe Ala His Leu Ala Ser
 195 200 205
 Glu Phe Lys Asn Pro Glu Arg Asp Phe Pro Arg Ala Leu Met Ile Gly
 210 215 220
 Leu Leu Leu Ala Gly Leu Val Tyr Trp Gly Cys Thr Val Val Val Leu
 225 230 235 240
 His Phe Asp Ala Tyr Gly Glu Lys Met Ala Ala Ala Ala Ser Leu Pro
 245 250 255

OPA15135_Sequence Listing

Lys Ile Val Val Gln Leu Phe Gly Val Gly Ala Leu Trp Ile Ala Cys
260 265 270

Val Ile Gly Tyr Leu Ala Cys Phe Ala Ser Leu Asn Ile Tyr Ile Gln
275 280 285

Ser Phe Ala Arg Leu Val Trp Ser Gln Ala Gln His Asn Pro Asp His
290 295 300

Tyr Leu Ala Arg Leu Ser Ser Arg His Ile Pro Asn Asn Ala Leu Asn
305 310 315 320

Ala Val Leu Gly Cys Cys Val Val Ser Thr Leu Val Ile His Ala Leu
325 330 335

Glu Ile Asn Leu Asp Ala Leu Ile Ile Tyr Ala Asn Gly Ile Phe Ile
340 345 350

Met Ile Tyr Leu Leu Cys Met Leu Ala Gly Cys Lys Leu Leu Gln Gly
355 360 365

Arg Tyr Arg Leu Leu Ala Val Val Gly Gly Leu Leu Cys Val Leu Leu
370 375 380

Leu Ala Met Val Gly Trp Lys Ser Leu Tyr Ala Leu Ile Met Leu Ala
385 390 395 400

Gly Leu Trp Leu Leu Leu Pro Lys Arg Lys Thr Pro Glu Asn Gly Ile
405 410 415

Thr Thr

<210> 2
<211> 1257
<212> DNA
<213> E.coli yjeH

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agcctgtggg cgtggcccg tttgattatc ttagtggtcc cgattgcat tgtgtttgcg 180
attctgggtc gccactatcc cagcgcaggc ggcgtcgcgc acttcgtcgg tatggcgttt 240
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cctgccgcac tacaaattgc cgccgggttc ggccaggcga tgtttggctg gcatagctgg 360
caactgttgt tggcagaact cggtagctg gcgctggtgt ggtatatcgg tactcgcggt 420
gccagttcca gtgctaactt acaaaccgtt attgccggac ttatcgtcgc gctgattgtc 480
gctatctggt gggcgggcga tatcaaacct gcgaatatcc cttttccggc acctggtaat 540
atcgaactta ccgggttatt tgctgcgtta tcagtgatgt tctggtgttt tgtcgggtctg 600
gaggcatttg ccatctcgc ctcggaattt aaaaatccag agcgtgattt tcctcgtgct 660
ttgatgattg gtctgctgct ggcaggatta gtctactggg gctgtacggt agtcgtctta 720
cacttcgacg cctatggtga aaaaatggcg gcggcagcat cgcttcaaaa aattgtagtg 780
cagttgttcg gtgtaggagc gttatggatt gcctgcgtga ttggctatct ggcctgcttt 840

OPA15135_Sequence Listing

gccagtctca acatttatat acagagcttc gcccgcttg tctggctgca ggcgcaacat 900
aatcctgacc actacctggc acgcctctct tctcgccata tcccgaataa tgccctcaat 960
gcggtgctcg gctgctgtgt ggtgagcact ttggtgattc atgctttaga gatcaatctg 1020
gacgctctta ttatttatgc caatggcatc tttattatga tttatctgtt atgcatgctg 1080
gcaggctgta aattattgca aggacgttat cgactactgg cgggtggttg cgggctgtta 1140
tgcgttctgt tactggcaat ggtcggctgg aaaagtctct atgcgctgat catgctggcg 1200
gggttatggc tgttgctgcc aaaacgaaaa acgccgaaa atggcataac cacataa 1257

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<211> 386
<212> PRT
<213> E.coli metB

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Asp Glu Gln Tyr Gly Cys Val Val Pro Pro Ile His Leu Ser Ser Thr
20 25 30
Tyr Asn Phe Thr Gly Phe Asn Glu Pro Arg Ala His Asp Tyr Ser Arg
35 40 45
Arg Gly Asn Pro Thr Arg Asp Val Val Gln Arg Ala Leu Ala Glu Leu
50 55 60
Glu Gly Gly Ala Gly Ala Val Leu Thr Asn Thr Gly Met Ser Ala Ile
65 70 75 80
His Leu Val Thr Thr Val Phe Leu Lys Pro Gly Asp Leu Leu Val Ala
85 90 95
Pro His Asp Cys Tyr Gly Gly Ser Tyr Arg Leu Phe Asp Ser Leu Ala
100 105 110
Lys Arg Gly Cys Tyr Arg Val Leu Phe Val Asp Gln Gly Asp Glu Gln
115 120 125
Ala Leu Arg Ala Ala Leu Ala Glu Lys Pro Lys Leu Val Leu Val Glu
130 135 140
Ser Pro Ser Asn Pro Leu Leu Arg Val Val Asp Ile Ala Lys Ile Cys
145 150 155 160
His Leu Ala Arg Glu Val Gly Ala Val Ser Val Val Asp Asn Thr Phe
165 170 175
Leu Ser Pro Ala Leu Gln Asn Pro Leu Ala Leu Gly Ala Asp Leu Val
180 185 190
Leu His Ser Cys Thr Lys Tyr Leu Asn Gly His Ser Asp Val Val Ala
195 200 205
Gly Val Val Ile Ala Lys Asp Pro Asp Val Val Thr Glu Leu Ala Trp
210 215 220
Trp Ala Asn Asn Ile Gly Val Thr Gly Gly Ala Phe Asp Ser Tyr Leu
225 230 235 240
Leu Leu Arg Gly Leu Arg Thr Leu Val Pro Arg Met Glu Leu Ala Gln
245 250 255

OPA15135_Sequence Listing

Arg Asn Ala Gln Ala Ile Val Lys Tyr Leu Gln Thr Gln Pro Leu Val
260 265 270
Lys Lys Leu Tyr His Pro Ser Leu Pro Glu Asn Gln Gly His Glu Ile
275 280 285
Ala Ala Arg Gln Gln Lys Gly Phe Gly Ala Met Leu Ser Phe Glu Leu
290 295 300
Asp Gly Asp Glu Gln Thr Leu Arg Arg Phe Leu Gly Gly Leu Ser Leu
305 310 315 320
Phe Thr Leu Ala Glu Ser Leu Gly Gly Val Glu Ser Leu Ile Ser His
325 330 335
Ala Ala Thr Met Thr His Ala Gly Met Ala Pro Glu Ala Arg Ala Ala
340 345 350
Ala Gly Ile Ser Glu Thr Leu Leu Arg Ile Ser Thr Gly Ile Glu Asp
355 360 365
Gly Glu Asp Leu Ile Ala Asp Leu Glu Asn Gly Phe Arg Ala Ala Asn
370 375 380
Lys Gly
385

<210> 4
<211> 1161
<212> DNA
<213> E.coli metB

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ggttgcgttg tcccaccgat ccattcttcc agcacctata actttaccgg atttaatgaa 120
ccgcgcgcgc atgattactc gcgtcgcggc aaccacaacgc gcgatgtggt tcagcgtgcg 180
ctggcagaac tggaaggttg tgctggtgca gtacttacta ataccggcat gtccgcgatt 240
cacctggtaa cgaccgtctt ttgaaacct ggcgatctgc tggttgcgcc gcacgactgc 300
tacggcggtg gctatgcct gttcgacagt ctggcgaaac gcggttgcta tcgcgtgttg 360
tttgttgatc aaggcgatga acaggcatta cgggcagcgc tggcagaaaa acccaactg 420
gtactggtag aaagcccaag taatccattg ttacgcgtcg tggatattgc gaaaatctgc 480
catctggcaa gggaagtcgg ggcggtgagc gtggtggata acaccttctt aagcccggca 540
ttacaaaatc cgctggcatt aggtgccgat ctggtgttgc attcatgcac gaaatatctg 600
aacggtcact cagacgtagt ggccggcgtg gtgattgcta aagaccgga cgttgtcact 660
gaactggcct ggtgggcaaa caatattggc gtgacgggcg gcgcgtttga cagctatctg 720
ctgctacgtg ggttgcgaaac gctggtgccg cgtatggagc tggcgcagcg caacgcgcag 780
gcgattgtga aatacctgca aaccagccg ttggtgaaaa aactgtatca cccgtcgttg 840
ccggaaaatc aggggcatga aattgccgcg cgccagcaaa aaggctttgg cgcaatgttg 900
agttttgaac tggatggcga tgagcagacg ctgcgtcggt tcctgggagg gctgtcgttg 960
tttacgctgg cggaatcatt agggggagtg gaaagtttaa tctctcacgc cgcaaccatg 1020

OPA15135_Sequence Listing

acacatgcag gcatggcacc agaagcgcgt gctgccgccg ggatctccga gacgctgctg 1080
 cgtatctcca ccggtattga agatggcgaa gatttaattg ccgacctgga aaatggcttc 1140
 cgggctgcaa acaaggggta a 1161

<210> 5
 <211> 310
 <212> PRT
 <213> E.coli thrB

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

OPA15135_Sequence Listing

Gln Asn Gln Glu Gly Phe Val His Ile Cys Arg Leu Asp Thr Ala Gly
 290 295 300

Ala Arg Val Leu Glu Asn
 305 310

<210> 6
 <211> 933
 <212> DNA
 <213> E,coli thrB

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 ggggcggcgg tgacacctgt tgatggtgca ttgctcggag atgtagtcac ggttgaggcg 120
 gcagagacat tcagtctcaa caacctcgga cgctttgccg ataagctgcc gtcagaacca 180
 cgggaaaata tcgtttatca gtgctgggag cgtttttgcc aggaactggg taagcaaatt 240
 ccagtggcga tgacctgga aaagaatatg ccgatcgggtt cgggcttagg ctccagtgcc 300
 tgttcgggtg tcgcggcgct gatggcgatg aatgaacact gcggcaagcc gcttaatgac 360
 actcgtttgc tggctttgat gggcgagctg gaaggccgta tctccggcag cattcattac 420
 gacaacgtgg caccgtgttt tctcggtggt atgcagttga tgatcgaaga aaacgacatc 480
 atcagccagc aagtgccagg gtttgatgag tggctgtggg tgctggcgta tccggggatt 540
 aaagtctcga cggcagaagc cagggtatt ttaccggcgc agtatcgccg ccaggattgc 600
 attgcgcacg ggcgacatct ggcaggcttc attcacgcct gctattcccg tcagcctgag 660
 cttgccgcga agctgatgaa agatgttatc gctgaaccct accgtgaacg gttactgcc 720
 ggcttccggc aggcgcggca ggcggtcgcg gaaatcggcg cggtagcgag cggtatctcc 780
 ggctccggcc cgacctgtt cgctctgtgt gacaagccgg aaaccgcca gcgcgttgcc 840
 gactggttgg gtaagaacta cctgcaaat caggaagggt ttgttcatat ttgccggctg 900
 gatacggcgg gcgcacgagt actggaaaac taa 933

<210> 7
 <211> 309
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> meta

<400> 7
 Met Pro Ile Arg Val Pro Asp Glu Leu Pro Ala Val Asn Phe Leu Arg
 1 5 10 15
 Glu Glu Asn Val Phe Val Met Thr Thr Ser Arg Ala Ser Gly Gln Glu
 20 25 30
 Ile Arg Pro Leu Lys Val Leu Ile Leu Asn Leu Met Pro Lys Lys Ile
 35 40 45
 Glu Thr Glu Asn Gln Phe Leu Arg Leu Leu Ser Asn Ser Pro Leu Gln
 50 55 60

OPA15135_Sequence Listing

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

<210> 8
<211> 933
<212> DNA
<213> Artificial Sequence
<220>
<223> meta

<400> 8
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ggggcgggcgg tgacacctgt tgatggtgca ttgctcggag atgtagtcac ggttgaggcg 120
gcagagacat tcagtctcaa caacctcgga cgctttgccg ataagctgcc gtcagaacca 180
cgggaaaata tcgtttatca gtgctgggag cgtttttgcc aggaactggg taagcaaatt 240
ccagtggcga tgacctgga aaagaatatg ccgatcggtt cgggcttagg ctccagtgcc 300

OPA15135_Sequence Listing

tgttcgggtgg tcgcggcgct gatggcgatg aatgaacact gcggcaagcc gcttaatgac	360
actcgtttgc tggctttgat gggcgagctg gaaggccgta tctccggcag cattcattac	420
gacaacgtgg caccgtgttt tctcgggtgg atgcagttga tgatcgaaga aaacgacatc	480
atcagccagc aagtgccagg gtttcatgag tggctgtggg tgctggcgta tccggggatt	540
aaagtctcga cggcagaagc cagggctatt ttaccggcgc agtatcgccg ccaggattgc	600
attgcgcacg ggcgacatct ggcaggcttc attcacgcct gctattcccg tcagcctgag	660
cttgccgcga agctgatgaa agatgttatc gctgaaccct accgtgaacg gttactgcc	720
ggcttccggc aggcgcggca ggcggtcgcg gaaatcggcg cggtagcgag cggtatctcc	780
ggctccggcc cgacctgtt cgctctgtgt gacaagccgg aaaccgcca gcgcgttgcc	840
gactggttgg gtaagaacta cctgcaaaat caggaagggt ttgttcatat ttgccggctg	900
gatacggcgg gcgcacgagt actggaaaac taa	933

<210> 9
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> yjeH primer

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<210> 10
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> yjeH primer

<400> 10	
aagctttatg tggttatgcc at	22

<210> 11
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> yjeH gene primer

<400> 11	
ggtaccaggt cgactctaga ggatcccc	28

<210> 12
 <211> 23
 <212> DNA
 <213> Artificial Sequence

OPA15135_Sequence Listing

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<223>	yjeH gene primer		
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	ggtaccttat gtggttatgc cat		23
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<211>	70		
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<213>	Artificial Sequence		
<220>			
<223>	MetB primer		
<400>	13		
	ttacccttg tttgcagccc ggaagccatt ttccaggctg gcaattaaat catatgaata		60
	tcctccttag		70
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<211>	70		
<212>	DNA		
<213>	Artificial Sequence		
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<223>	metB primer		
<400>	14		
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	gagctgcttc		70
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<213>	Artificial Sequence		
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<223>	thrB primer		
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	gagctgcttc		70
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<213>	Artificial Sequence		
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<223>	thrB primer		
<400>	16		
	agacaaccga catcgctttc aacattggcg accggagccg ggaaggcaaa catatgaata		60
	tcctccttag		70

OPA15135_Sequence Listing

<210>	17	
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<212>	DNA	
<213>	Artificial Sequence	
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<223>	metaA primer	
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<210>	18	
<211>	34	
<212>	DNA	
<213>	Artificial Sequence	
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<223>	metaA primer	
<400>	18	
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<210>	19	
<211>	44	
<212>	DNA	
<213>	Artificial Sequence	
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<223>	meta G111E mutagenesis primer	
<400>	19	
ttgtaactgg tgcgccgctg gaactggtgg gggttaatatga gtgc		44
<210>	20	
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gacatcatta aacccaccca gttccagcgg cgcaccagtt acaa		44
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tgtaactggt gcgccgctgg aacatgtggg gtttaatgat gtcg		44
<210>	22	
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OPA15135_Sequence Listing

<213> Artificial Sequence

<220>

<223> metaA EH mutagenesis primer

<400> 22
cgacatcatt aaaccccaca tgttccagcg gcgcaccagt taca 44

<210> 23
<211> 29
<212> DNA
<213> Artificial Sequence

<220>

<223> metaA EH primer

<400> 23
aattgatatc atgccgattc gtgtgccgg 29

<210> 24
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<212> DNA
<213> Artificial Sequence

<220>

<223> metaA EH primer

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aattaagcct gctgaggtac gtttcgg 27

<210> 25
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<213> Artificial Sequence

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<223> metaA WT primer

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cagcaggtga ataaatttta ttc 23

<210> 26
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> metaA WT primer

<400> 26
cgcgaatgga agctgtttcc 20

<210> 27
<211> 42
<212> DNA
<213> Artificial Sequence

<220>

OPA15135_Sequence Listing

<223> pKD3 primer

<400> 27
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<210> 28
 <211> 43
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> pKD3 primer

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<210> 29
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> ppc 2 copy primer

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<210> 30
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> ppc 2 copy primer

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<210> 31
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> ppc 2 copy primer

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 gccggagctc tgtcggatgc gatacttgcg c 31

<210> 32
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> ppc 2 copy primer

OPA15135_Sequence Listing

<400>	32		
gaagggtacc agaaaaccct cgcgcaaaag			30
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<212>	DNA		
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<223>	aspC 2 copy primer		
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tccgagctca taagcgtagc gcatcaggca			30
<210>	34		
<211>	30		
<212>	DNA		
<213>	Artificial Sequence		
<220>			
<223>	aspC 2 copy primer		
<400>	34		
tccgagctcg tccacctatg ttgactacat			30
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<211>	27		
<212>	DNA		
<213>	Artificial Sequence		
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<223>	asd 2 copy primer		
<400>	35		
ccggaattcc caggagagca ataagca			27
<210>	36		
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<213>	Artificial Sequence		
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<223>	asd 2 copy primer		
<400>	36		
ctagtctaga tgctctatTT aactcccG			28
<210>	37		
<211>	28		
<212>	DNA		
<213>	Artificial Sequence		
<220>			
<223>	asd 2 copy primer		
<400>	37		
ctagtctaga ccaggagagc aataagca			28

OPA15135_Sequence Listing

<210>	38	
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<212>	DNA	
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<223>	asd 2 copy primer	
<400>	38	
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<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	icd promoter primer	
<400>	39	
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<210>	40	
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<212>	DNA	
<213>	Artificial Sequence	
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OPA15135_Sequence Listing

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OPA15135_Sequence Listing

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OPA15135_Sequence Listing

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