SEQUENCE LISTING

<110> Janssen Pharmaceuticals, Inc.
GlaxoSmithKline Biologicals S.A.

<120> Methods of Producing Bioconjugates of E. coli O-Antigen Polysaccharides, Compositions Thereof, and Methods of Use Thereof

<130> 004852.11612/128WO1 (CRU6009WOPCT1; VB66734P)

<150> US62/819,762

<151> 2019-03-18

<160> 19

<170> PatentIn version 3.5

<210> 1
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<212> PRT
<213> Artificial Sequence

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<223> glycosylation consensus sequence

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<222> (2)..(2)
<223> any amino acid residue except Pro

<220>
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<222> (3)..(3)
<223> Ser orThr

<400> 1

Asn Xaa Xaa
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<210> 2
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<222> (1)..(1)
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<223> any amino acid residue except Pro

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<222> (5)..(5)
<223> Ser or Thr

<400> 2

Xaa Xaa Asn Xaa Xaa
1 5

<210> 3
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<223> EPA carrier protein comprising 4 glycosylation consensus sequences (EPA-4)

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Gly Ser Gly Gly Gly Asp Gln Asn Ala Thr Gly Ser Gly Gly Gly Lys
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Leu Ala Glu Glu Ala Phe Asp Leu Trp Asn Glu Cys Ala Lys Ala Cys
20 25 30

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

Pro Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met
50 55 60

Val Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala
65 70 75 80

```
Leu Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val
                85                  90                  95

Glu Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly
            100                 105                 110

Ser Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser
        115                 120                 125

Asn Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser
    130                 135                 140

His Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala
145                 150                 155                 160

Lys Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn
                165                 170                 175

Glu Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val
            180                 185                 190

Met Ala Gln Ala Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala
        195                 200                 205

Ser Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn
    210                 215                 220

Tyr Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys
225                 230                 235                 240

Ile Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile
                245                 250                 255

Lys Asp Asn Asn Asn Ser Thr Pro Thr Val Ile Ser His Arg Leu His
            260                 265                 270

Phe Pro Glu Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln Ala Cys
        275                 280                 285

His Leu Pro Leu Glu Ala Phe Thr Arg His Arg Gln Pro Arg Gly Trp
```

```
    290                 295                 300

Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val Ala Leu
305                 310                 315                 320

Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val Ile Arg
                325                 330                 335

Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile
            340                 345                 350

Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala
        355                 360                 365

Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly
    370                 375                 380

Ala Ala Ser Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala Lys Asp
385                 390                 395                 400

Gln Asn Arg Thr Lys Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp
                405                 410                 415

Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp
            420                 425                 430

Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Thr Gln Asn Trp Thr Val
        435                 440                 445

Glu Arg Leu Leu Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val
    450                 455                 460

Phe Val Gly Tyr His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val
465                 470                 475                 480

Phe Gly Gly Val Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg
                485                 490                 495

Gly Phe Tyr Ile Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln
            500                 505                 510
```

```
Asp Gln Glu Pro Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu
        515                 520                 525

Arg Val Tyr Val Pro Arg Trp Ser Leu Pro Gly Phe Tyr Arg Thr Gly
    530                 535                 540

Leu Thr Leu Ala Ala Pro Glu Ala Ala Gly Glu Val Glu Arg Leu Ile
545                 550                 555                 560

Gly His Pro Leu Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu
                565                 570                 575

Glu Gly Gly Arg Val Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr
            580                 585                 590

Val Val Ile Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly
        595                 600                 605

Asp Leu Asp Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala
    610                 615                 620

Leu Pro Asp Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Leu
625                 630                 635                 640

Lys Leu Gly Ser Gly Gly Gly Asp Gln Asn Ala Thr
                645                 650
```

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<211> 421
<212> PRT
<213> Artificial Sequence

<220>
<223> O4 GtrS amino acid sequence

<400> 4

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Met Asn Asn Leu Ile Met Asn Asn Trp Cys Lys Leu Ser Ile Phe Ile
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Ile Ala Phe Ile Leu Leu Trp Leu Arg Arg Pro Asp Ile Leu Thr Asn
            20                  25                  30
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```
Ala Gln Phe Trp Ala Glu Asp Ser Val Phe Trp Tyr Lys Asp Ala Tyr
        35                  40                  45

Glu Asn Gly Phe Leu Ser Ser Leu Thr Thr Pro Arg Asn Gly Tyr Phe
    50                  55                  60

Gln Thr Val Ser Thr Phe Ile Val Gly Leu Thr Ala Leu Leu Asn Pro
65                  70                  75                  80

Asp Tyr Ala Pro Phe Val Ser Asn Phe Phe Gly Ile Met Ile Arg Ser
                85                  90                  95

Val Ile Ile Trp Phe Leu Phe Thr Glu Arg Phe Asn Phe Leu Thr Leu
            100                 105                 110

Thr Thr Arg Ile Phe Leu Ser Ile Tyr Phe Leu Cys Met Pro Gly Leu
        115                 120                 125

Asp Glu Val His Ala Asn Ile Thr Asn Ala His Trp Tyr Leu Ser Leu
    130                 135                 140

Tyr Val Ser Met Ile Leu Ile Ala Arg Asn Pro Ser Ser Lys Ser Trp
145                 150                 155                 160

Arg Phe His Asp Ile Phe Phe Ile Leu Leu Ser Gly Leu Ser Gly Pro
                165                 170                 175

Phe Ile Ile Phe Ile Leu Ala Ala Ser Cys Phe Lys Phe Ile Asn Asn
            180                 185                 190

Cys Lys Asp His Ile Ser Val Arg Ser Phe Ile Asn Phe Tyr Leu Arg
        195                 200                 205

Gln Pro Tyr Ala Leu Met Ile Val Cys Ala Leu Ile Gln Gly Thr Ser
    210                 215                 220

Ile Ile Leu Thr Phe Asn Gly Thr Arg Ser Ser Ala Pro Leu Gly Phe
225                 230                 235                 240

Ser Phe Asp Val Ile Ser Ser Ile Ile Ser Ser Asn Ile Phe Leu Phe
```

245 250 255

Thr Phe Val Pro Trp Asp Ile Ala Lys Ala Gly Trp Asp Asn Leu Leu
260 265 270

Leu Ser Tyr Phe Leu Ser Val Ser Ile Leu Ser Cys Ala Ala Phe Val
275 280 285

Phe Val Lys Gly Thr Trp Arg Met Lys Val Phe Ala Thr Leu Pro Leu
290 295 300

Leu Ile Ile Ile Phe Ser Met Ala Lys Pro Gln Leu Thr Asp Ser Ala
305 310 315 320

Pro Gln Leu Pro Thr Leu Ile Asn Gly Gln Gly Ser Arg Tyr Phe Val
325 330 335

Asn Ile His Ile Ala Ile Phe Ser Leu Leu Cys Val Tyr Leu Leu Glu
340 345 350

Cys Val Arg Gly Lys Val Ala Thr Leu Phe Ser Lys Ile Tyr Leu Thr
355 360 365

Ile Leu Leu Phe Val Met Gly Cys Leu Asn Phe Val Ile Thr Pro Leu
370 375 380

Pro Asn Met Asn Trp Arg Glu Gly Ala Thr Leu Ile Asn Asn Ala Lys
385 390 395 400

Thr Gly Asp Val Ile Ser Ile Gln Val Leu Pro Pro Gly Leu Thr Leu
405 410 415

Glu Leu Arg Lys Lys
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<210> 5
<211> 1266
<212> DNA
<213> Artificial Sequence

<220>
<223> Example O4 gtrS nucleic acid sequence

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gttttctggt ataaggacgc ctatgagaac ggattcttaa gttcactaac aacgcctagg 180

aatgggtatt tccagactgt ttctacattt atagttggtc tgactgcttt attaaatcca 240

gattatgcac cttttgtttc taatttttt ggcataatga ttcgctcagt aattatatgg 300

tttttattta cagaaagatt caacttcctc acattgacta ctaggatttt cttatctatt 360

tattttctat gcatgcctgg attggatgaa gttcatgcaa atataacaaa tgcacattgg 420

tatttgtcat tatatgtatc aatgatcctg atagctcgca atccaagttc aaaatcatgg 480

aggtttcatg atatattctt tatcttgcta tccgggctca gtggcccatt tataattttc 540

attttagcag cttcatgctt taaatttata aataattgta aagatcatat tagtgtaaga 600

tctttcataa atttctactt gcgtcagcca tacgcattaa tgattgtttg cgctttaatt 660

caaggaactt ctataattct aactttcaat ggcacacgtt cctcagcacc gctaggattc 720

agttttgatg tgatttcgtc tattatatca tcgaatattt ttttatttac atttgtccca 780

tgggatattg caaaggctgg gtgggataat ttactgttat cttatttttt gtctgtttcg 840

attttgtcgt gtgcggcctt tgtttttgtt aaaggtacgt ggcgaatgaa agtatttgca 900

actttaccat tgctaattat aatattttca atggcaaaac cacaattgac agactcggca 960

cctcaattgc caacacttat taatgggcaa ggttcaagat acttcgtaaa tatacatatt 1020

gcgatattct ctttgctatg tgtttactta cttgagtgcg tcaggggggaa agtggcaact 1080

ttattttcca aaatatactt aacaattttg ctattcgtga tgggatgttt gaattttgtt 1140

atcaccccac tcccaaacat gaactggagg gaaggtgcta ctttgattaa taatgcaaaa 1200

actggtgatg tcatttcgat tcaagtgcta ccacctggcc taacacttga actaaggaaa 1260

aaataa 1266

<210> 6
<211> 713
<212> PRT
<213> Artificial Sequence

<220>
<223> Example PglB sequence ('wild-type')

<400> 6

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Met Leu Lys Lys Glu Tyr Leu Lys Asn Pro Tyr Leu Val Leu Phe Ala
1               5                  10                  15

Met Ile Ile Leu Ala Tyr Val Phe Ser Val Phe Cys Arg Phe Tyr Trp
            20                  25                  30

Val Trp Trp Ala Ser Glu Phe Asn Glu Tyr Phe Phe Asn Asn Gln Leu
        35                  40                  45

Met Ile Ile Ser Asn Asp Gly Tyr Ala Phe Ala Glu Gly Ala Arg Asp
    50                  55                  60

Met Ile Ala Gly Phe His Gln Pro Asn Asp Leu Ser Tyr Tyr Gly Ser
65                  70                  75                  80

Ser Leu Ser Ala Leu Thr Tyr Trp Leu Tyr Lys Ile Thr Pro Phe Ser
                85                  90                  95

Phe Glu Ser Ile Ile Leu Tyr Met Ser Thr Phe Leu Ser Ser Leu Val
            100                 105                 110

Val Ile Pro Thr Ile Leu Leu Ala Asn Glu Tyr Lys Arg Pro Leu Met
        115                 120                 125

Gly Phe Val Ala Ala Leu Leu Ala Ser Ile Ala Asn Ser Tyr Tyr Asn
    130                 135                 140

Arg Thr Met Ser Gly Tyr Tyr Asp Thr Asp Met Leu Val Ile Val Leu
145                 150                 155                 160

Pro Met Phe Ile Leu Phe Phe Met Val Arg Met Ile Leu Lys Lys Asp
                165                 170                 175

Phe Phe Ser Leu Ile Ala Leu Pro Leu Phe Ile Gly Ile Tyr Leu Trp
            180                 185                 190

Trp Tyr Pro Ser Ser Tyr Thr Leu Asn Val Ala Leu Ile Gly Leu Phe
        195                 200                 205
```

```
Leu Ile Tyr Thr Leu Ile Phe His Arg Lys Glu Lys Ile Phe Tyr Ile
    210                 215                 220

Ala Val Ile Leu Ser Ser Leu Thr Leu Ser Asn Ile Ala Trp Phe Tyr
225                 230                 235                 240

Gln Ser Ala Ile Ile Val Ile Leu Phe Ala Leu Phe Ala Leu Glu Gln
                245                 250                 255

Lys Arg Leu Asn Phe Met Ile Ile Gly Ile Leu Gly Ser Ala Thr Leu
            260                 265                 270

Ile Phe Leu Ile Leu Ser Gly Gly Val Asp Pro Ile Leu Tyr Gln Leu
        275                 280                 285

Lys Phe Tyr Ile Phe Arg Ser Asp Glu Ser Ala Asn Leu Thr Gln Gly
    290                 295                 300

Phe Met Tyr Phe Asn Val Asn Gln Thr Ile Gln Glu Val Glu Asn Val
305                 310                 315                 320

Asp Leu Ser Glu Phe Met Arg Arg Ile Ser Gly Ser Glu Ile Val Phe
                325                 330                 335

Leu Phe Ser Leu Phe Gly Phe Val Trp Leu Leu Arg Lys His Lys Ser
            340                 345                 350

Met Ile Met Ala Leu Pro Ile Leu Val Leu Gly Phe Leu Ala Leu Lys
        355                 360                 365

Gly Gly Leu Arg Phe Thr Ile Tyr Ser Val Pro Val Met Ala Leu Gly
    370                 375                 380

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

Ser Gln Leu Thr Ser Asn Val Cys Ile Val Phe Ala Thr Ile Leu Thr
                405                 410                 415

Leu Ala Pro Val Phe Ile His Ile Tyr Asn Tyr Lys Ala Pro Thr Val
```

```
            420                 425                 430

Phe Ser Gln Asn Glu Ala Ser Leu Leu Asn Gln Leu Lys Asn Ile Ala
        435                 440                 445

Asn Arg Glu Asp Tyr Val Val Thr Trp Trp Asp Tyr Gly Tyr Pro Val
    450                 455                 460

Arg Tyr Tyr Ser Asp Val Lys Thr Leu Val Asp Gly Gly Lys His Leu
465                 470                 475                 480

Gly Lys Asp Asn Phe Phe Pro Ser Phe Ala Leu Ser Lys Asp Glu Gln
                485                 490                 495

Ala Ala Ala Asn Met Ala Arg Leu Ser Val Glu Tyr Thr Glu Lys Ser
            500                 505                 510

Phe Tyr Ala Pro Gln Asn Asp Ile Leu Lys Thr Asp Ile Leu Gln Ala
        515                 520                 525

Met Met Lys Asp Tyr Asn Gln Ser Asn Val Asp Leu Phe Leu Ala Ser
    530                 535                 540

Leu Ser Lys Pro Asp Phe Lys Ile Asp Thr Pro Lys Thr Arg Asp Ile
545                 550                 555                 560

Tyr Leu Tyr Met Pro Ala Arg Met Ser Leu Ile Phe Ser Thr Val Ala
                565                 570                 575

Ser Phe Ser Phe Ile Asn Leu Asp Thr Gly Val Leu Asp Lys Pro Phe
            580                 585                 590

Thr Phe Ser Thr Ala Tyr Pro Leu Asp Val Lys Asn Gly Glu Ile Tyr
        595                 600                 605

Leu Ser Asn Gly Val Val Leu Ser Asp Asp Phe Arg Ser Phe Lys Ile
    610                 615                 620

Gly Asp Asn Val Val Ser Val Asn Ser Ile Val Glu Ile Asn Ser Ile
625                 630                 635                 640
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```
Lys Gln Gly Glu Tyr Lys Ile Thr Pro Ile Asp Asp Lys Ala Gln Phe
                645                 650                 655

Tyr Ile Phe Tyr Leu Lys Asp Ser Ala Ile Pro Tyr Ala Gln Phe Ile
            660                 665                 670

Leu Met Asp Lys Thr Met Phe Asn Ser Ala Tyr Val Gln Met Phe Phe
        675                 680                 685

Leu Gly Asn Tyr Asp Lys Asn Leu Phe Asp Leu Val Ile Asn Ser Arg
    690                 695                 700

Asp Ala Lys Val Phe Lys Leu Lys Ile
705                 710
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<210> 7
<211> 120
<212> PRT
<213> Escherichia coli

<400> 7

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Met Leu Lys Leu Phe Ala Lys Tyr Thr Ser Ile Gly Val Leu Asn Thr
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Leu Ile His Trp Val Val Phe Gly Val Cys Ile Tyr Val Ala His Thr
            20                  25                  30

Asn Gln Ala Leu Ala Asn Phe Ala Gly Phe Val Val Ala Val Ser Phe
        35                  40                  45

Ser Phe Phe Ala Asn Ala Lys Phe Thr Phe Lys Ala Ser Thr Thr Thr
    50                  55                  60

Met Arg Tyr Met Leu Tyr Val Gly Phe Met Gly Thr Leu Ser Ala Thr
65                  70                  75                  80

Val Gly Trp Ala Ala Asp Arg Cys Ala Leu Pro Pro Met Ile Thr Leu
                85                  90                  95

Val Thr Phe Ser Ala Ile Ser Leu Val Cys Gly Phe Val Tyr Ser Lys
            100                 105                 110
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Phe Ile Val Phe Arg Asp Ala Lys
        115   120

<210> 8
<211> 306
<212> PRT
<213> Escherichia coli

<400> 8

Met Lys Ile Ser Leu Val Val Pro Val Phe Asn Glu Glu Glu Ala Ile
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Pro Ile Phe Tyr Lys Thr Val Arg Glu Phe Glu Glu Leu Lys Ser Tyr
            20   25   30

Glu Val Glu Ile Val Phe Ile Asn Asp Gly Ser Lys Asp Ala Thr Glu
        35   40   45

Ser Ile Ile Asn Ala Leu Ala Val Ser Asp Pro Leu Val Val Pro Leu
    50   55   60

Ser Phe Thr Arg Asn Phe Gly Lys Glu Pro Ala Leu Phe Ala Gly Leu
65   70   75   80

Asp His Ala Thr Gly Asp Ala Ile Ile Pro Ile Asp Val Asp Leu Gln
                85   90   95

Asp Pro Ile Glu Val Ile Pro His Leu Ile Glu Lys Trp Gln Ala Gly
            100   105   110

Ala Asp Met Val Leu Ala Lys Arg Ser Asp Arg Ser Thr Asp Gly Arg
        115   120   125

Leu Lys Arg Lys Thr Ala Glu Trp Phe Tyr Lys Leu His Asn Lys Ile
    130   135   140

Ser Asn Pro Lys Ile Glu Glu Asn Val Gly Asp Phe Arg Leu Met Ser
145   150   155   160

Arg Asp Val Val Glu Asn Ile Lys Leu Met Pro Glu Arg Asn Leu Phe

```
                165                 170                 175

Met Lys Gly Ile Leu Ser Trp Val Gly Gly Lys Thr Asp Ile Val Glu
            180                 185                 190

Tyr Val Arg Ala Glu Arg Ile Ala Gly Asp Thr Lys Phe Asn Gly Trp
        195                 200                 205

Lys Leu Trp Asn Leu Ala Leu Glu Gly Ile Thr Ser Phe Ser Thr Phe
    210                 215                 220

Pro Leu Arg Ile Trp Thr Tyr Ile Gly Leu Val Val Ala Ser Val Ala
225                 230                 235                 240

Phe Ile Tyr Gly Ala Trp Met Ile Leu Asp Thr Ile Ile Phe Gly Asn
                245                 250                 255

Ala Val Arg Gly Tyr Pro Ser Leu Leu Val Ser Ile Leu Phe Leu Gly
            260                 265                 270

Gly Ile Gln Met Ile Gly Ile Gly Val Leu Gly Glu Tyr Ile Gly Arg
        275                 280                 285

Thr Tyr Ile Glu Thr Lys Lys Arg Pro Lys Tyr Ile Ile Lys Arg Val
    290                 295                 300

Lys Lys
305
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<210> 9
<211> 14440
<212> DNA
<213> Artificial Sequence

<220>
<223> example O4 rfb locus nucleotide sequence - O4-EPA production strain BVEC-L-00684f

<400> 9

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gttgacgaga ttgtggctgc agggatcaaa gaaatcctcc tggtaactca cgcgtccaag      180
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aacgcggtcg aaaaccactt cgacacctct tatgagttag aatcactcct tgagcagcgc 240

gtgaagcgtc aactgctggc ggaagtacag tccatctgtc cgccgggcgt gaccattatg 300

aacgtgcgtc agggcgaacc tttaggttta ggccactcca ttttgtgtgc gcgacctgcc 360

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gacccgctac gttacaacct tgctgccatg attgcacgtt tcaacgaaac gggccgcagc 480

caggtgctgg caaaacgtat gccgggtgac ctctctgaat actccgtcat ccagactaaa 540

gagccgctgg accgtgaggg taaagtcagc cgcattgttg aatttatcga aaaaccggat 600

cagccgcaga cgctggactc agacatcatg gccgtaggtc gctatgtgct ttctgccgat 660

atttggccgg aactggaacg tactcagcct ggtgcatggg gacgtattca gctgactgat 720

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ctgaaagaag gggcgaagtt ccgtaaaggt attgagaagc tgttaagcga ataatgaaaa 900

tctgaccgga tgtaacggtt gataagaaaa ttataacggc agtgaaaatt cgcagcaaaa 960

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acagcttacg cgccaagcag cccttattcc gcatccaaag catccagcga tcatttagtc 1800

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ttgtcttgcc tgactggcag gtgggcgtga aacgtatgct caacgaatta tttacgacta 3240

cggcaattta acaaattttt gcatctcgct catgatgcca gagcgggatg aattaaaagg 3300

aatggtgaaa tgaaaacgcg taaaggtatt attctggctg gtggttccgg cactcgtctt 3360

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ccacaggata caccgcgttt ccaacaattg ttgggggacg ggagtcagtg ggggcttaat 3540

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