SEQUENCE LISTING

<110> SYNTAXIN LIMITED

<120> CATIONIC NEUROTOXINS

<130> P40989WO

<150> GB 1312317.9
<151> 2013-07-09

<160> 10

<170> PatentIn version 3.5

<210> 1
<211> 3888
<212> DNA
<213> Clostridium botulinum

<400> 1
atgccattcg tcaacaagca attcaactac aaagacccag tcaacggcgt cgacatcgca 60

tacatcaaga ttccgaacgc cggtcaaatg cagccggtta aggcttttaa gatccacaac 120

aagatttggg ttatcccgga gcgtgacacc ttcacgaacc cggaagaagg cgatctgaac 180

ccgccaccgg aagcgaagca agtccctgtc agctactacg attcgacgta cctgagcacg 240

gataacgaaa aagataacta cctgaaaggt gtgaccaagc tgttcgaacg tatctacagc 300

acggatctgg gtcgcatgct gctgactagc attgttcgcg gtatcccgtt ctggggtggt 360

agcacgattg acaccgaact gaaggttatc gacactaact gcattaacgt tattcaaccg 420

gatggtagct atcgtagcga agagctgaat ctggtcatca ttggcccgag cgcagacatt 480

atccaattcg agtgcaagag ctttggtcac gaggttctga atctgacccg caatggctat 540

ggtagcaccc agtacattcg tttttcgccg gattttacct tcggctttga agagagcctg 600

gaggttgata ccaatccgtt gctgggtgcg ggcaaattcg ctaccgatcc ggctgtcacg 660

ctggcccatg aactgatcca cgcaggccac cgcctgtacg gcattgccat caacccaaac 720

cgtgtgttca aggttaatac gaatgcatac tacgagatga gcggcctgga agtcagcttc 780

gaagaactgc gcaccttcgg tggccatgac gctaaattca ttgacagctt gcaagagaat 840

gagttccgtc tgtactacta taacaaattc aaagacattg caagcacgtt gaacaaggcc 900

aaaagcatcg ttggtactac cgcgtcgttg cagtatatga agaatgtgtt taaagagaag 960

tacctgctgt ccgaggatac ctccggcaag tttagcgttg ataagctgaa gtttgacaaa 1020

ctgtacaaga tgctgaccga gatttacacc gaggacaact ttgtgaaatt cttcaaagtg 1080

ttgaatcgta aaacctatct gaattttgac aaagcggttt tcaagattaa catcgtgccg 1140

aaggtgaact acaccatcta tgacggtttt aacctgcgta acaccaacct ggcggcgaac 1200

tttaacggtc agaatacgga aatcaacaac atgaatttca cgaagttgaa gaacttcacg 1260

ggtctgttcg agttctataa gctgctgtgc gtgcgcggta tcatcaccag caaaaccaaa 1320

agcctggaca aaggctacaa caaggcgctg aatgacctgt gcattaaggt aaacaattgg 1380

gatctgttct tttcgccatc cgaagataat tttaccaacg acctgaacaa gggtgaagaa 1440

atcaccagcg atacgaatat tgaagcagcg gaagagaata tcagcctgga tctgatccag 1500

cagtactatc tgacctttaa cttcgacaat gaaccggaga acattagcat tgagaatctg 1560

agcagcgaca ttatcggtca gctggaactg atgccgaata tcgaacgttt cccgaacggc 1620

aaaaagtacg agctggacaa gtacactatg ttccattacc tgcgtgcaca ggagtttgaa 1680

cacggtaaaa gccgtatcgc gctgaccaac agcgttaacg aggccctgct gaacccgagc 1740

cgtgtctata ccttcttcag cagcgactat gttaagaaag tgaacaaagc cactgaggcc 1800

gcgatgttcc tgggctgggt ggaacagctg gtatatgact tcacggacga gacgagcgaa 1860

gtgagcacta ccgacaaaat tgctgatatt accatcatta tcccgtatat tggtccggca 1920

ctgaacattg gcaacatgct gtacaaagac gattttgtgg gtgccctgat cttctccggt 1980

gccgtgattc tgctggagtt cattccggag attgcgatcc cggtgttggg taccttcgcg 2040

ctggtgtcct acatcgcgaa taaggttctg acggttcaga ccatcgataa cgcgctgtcg 2100

aaacgtaatg aaaaatggga cgaggtttac aaatacattg ttacgaattg gctggcgaaa 2160

gtcaataccc agatcgacct gatccgtaag aaaatgaaag aggcgctgga gaatcaggcg 2220

gaggccacca aagcaattat caactaccaa tacaaccagt acacggaaga agagaagaat 2280

aacattaact tcaatatcga tgatttgagc agcaagctga atgaatctat caacaaagcg 2340

atgatcaata tcaacaagtt tttgaatcag tgtagcgttt cgtacctgat gaatagcatg 2400

attccgtatg gcgtcaaacg tctggaggac ttcgacgcca gcctgaaaga tgcgttgctg 2460

aaatacattt acgacaatcg tggtacgctg attggccaag ttgaccgctt gaaagacaaa 2520

gttaacaata ccctgagcac cgacatccca tttcaactga gcaagtatgt tgataatcaa 2580

cgtctgttga gcactttcac cgagtatatc aaaaacatca tcaatactag cattctgaac 2640

ctgcgttacg agagcaatca tctgattgat ctgagccgtt atgcaagcaa gatcaacatc 2700

ggtagcaagg tcaattttga cccgatcgat aagaaccaga tccagctgtt taatctggaa 2760

tcgagcaaaa ttgaggttat cctgaaaaac gccattgtct acaactccat gtacgagaat 2820

ttctccacca gcttctggat tcgcatcccg aaatacttca acagcattag cctgaacaac 2880

gagtatacta tcatcaactg tatggagaac aacagcggtt ggaaggtgtc tctgaactat 2940

ggtgagatca tttggacctt gcaggacacc caagagatca agcagcgcgt cgtgttcaag 3000

tactctcaaa tgatcaacat ttccgattac attaatcgtt ggatcttcgt gaccattacg 3060

aataaccgtc tgaataacag caagatttac atcaatggtc gcttgatcga tcagaaaccg 3120

attagcaacc tgggtaatat ccacgcaagc aacaacatta tgttcaaatt ggacggttgc 3180

cgcgataccc atcgttatat ctggatcaag tatttcaacc tgtttgataa agaactgaat 3240

gagaaggaga tcaaagattt gtatgacaac caatctaaca gcggcatttt gaaggacttc 3300

tggggcgatt atctgcaata cgataagccg tactatatgc tgaacctgta tgatccgaac 3360

aaatatgtgg atgtcaataa tgtgggtatt cgtggttaca tgtatttgaa gggtccgcgt 3420

ggcagcgtta tgacgaccaa catttacctg aactctagcc tgtaccgtgg tacgaaattc 3480

atcattaaga aatatgccag cggcaacaaa gataacattg tgcgtaataa cgatcgtgtc 3540

tacatcaacg tggtcgtgaa gaataaagag taccgtctgg cgaccaacgc ttcgcaggcg 3600

ggtgttgaga aaattctgag cgcgttggag atccctgatg tcggtaatct gagccaagtc 3660

gtggttatga agagcaagaa cgaccagggt atcactaaca agtgcaagat gaacctgcaa 3720

gacaacaatg gtaacgacat cggctttatt ggtttccacc agttcaacaa tattgctaaa 3780

ctggtagcga gcaattggta caatcgtcag attgagcgca gcagccgtac tttgggctgt 3840

agctgggagt ttatcccggt cgatgatggt tggggcgaac gtccgctg 3888

<210> 2
<211> 1296
<212> PRT
<213> Clostridium botulinum

<400> 2

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15

Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30

Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
85 90 95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110

Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
130 135 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145 150 155 160

Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
165 170 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe

180 185 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
195 200 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
210 215 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225 230 235 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
245 250 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
260 265 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
275 280 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
290 295 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
325 330 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
355 360 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
370 375 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385 390 395 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
405 410 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
420 425 430

Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys
435 440 445

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe

```
    450                 455                 460

Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465                 470                 475                 480

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
                485                 490                 495

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
            500                 505                 510

Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
        515                 520                 525

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
    530                 535                 540

Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
545                 550                 555                 560

His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
                565                 570                 575

Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
            580                 585                 590

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
        595                 600                 605

Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
    610                 615                 620

Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
625                 630                 635                 640

Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
                645                 650                 655

Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
            660                 665                 670

Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
        675                 680                 685

Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
    690                 695                 700

Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705                 710                 715                 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
```

725 730 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
740 745 750

Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
755 760 765

Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
770 775 780

Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785 790 795 800

Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
805 810 815

Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
820 825 830

Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
835 840 845

Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
850 855 860

Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865 870 875 880

Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
885 890 895

Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
900 905 910

Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
915 920 925

Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
930 935 940

Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn
945 950 955 960

Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
965 970 975

Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu
980 985 990

Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser

995 1000 1005

Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg
1010 1015 1020

Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln
1025 1030 1035

Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile
1040 1045 1050

Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
1055 1060 1065

Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu
1070 1075 1080

Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys
1085 1090 1095

Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met
1100 1105 1110

Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val
1115 1120 1125

Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val
1130 1135 1140

Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
1145 1150 1155

Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile
1160 1165 1170

Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn
1175 1180 1185

Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu
1190 1195 1200

Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser
1205 1210 1215

Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn
1220 1225 1230

Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly
1235 1240 1245

Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala

1250 1255 1260

Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu
1265 1270 1275

Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu
1280 1285 1290

Arg Pro Leu
1295

<210> 3
<211> 3888
<212> DNA
<213> Artificial Sequence

<220>
<223> Engineered BoNT/A1

<400> 3
atgccattcg tcaacaagca attcaactac aaagacccag tcaacggcgt cgacatcgca 60

tacatcaaga ttccgaacgc cggtcaaatg cagccggtta aggcttttaa gatccacaac 120

aagatttggg ttatcccgga gcgtgacacc ttcacgaacc cggaagaagg cgatctgaac 180

ccgccaccgg aagcgaagca agtccctgtc agctactacg attcgacgta cctgagcacg 240

gataacgaaa aagataacta cctgaaaggt gtgaccaagc tgttcgaacg tatctacagc 300

acggatctgg gtcgcatgct gctgactagc attgttcgcg gtatcccgtt ctggggtggt 360

agcacgattg acaccgaact gaaggttatc gacactaact gcattaacgt tattcaaccg 420

gatggtagct atcgtagcga agagctgaat ctggtcatca ttggcccgag cgcagacatt 480

atccaattcg agtgcaagag ctttggtcac gaggttctga atctgacccg caatggctat 540

ggtagcaccc agtacattcg tttttcgccg gattttacct tcggctttga agagagcctg 600

gaggttgata ccaatccgtt gctgggtgcg ggcaaattcg ctaccgatcc ggctgtcacg 660

ctggcccatg aactgatcca cgcaggccac cgcctgtacg gcattgccat caacccaaac 720

cgtgtgttca aggttaatac gaatgcatac tacgagatga gcggcctgga agtcagcttc 780

gaagaactgc gcaccttcgg tggccatgac gctaaattca ttgacagctt gcaagagaat 840

gagttccgtc tgtactacta taacaaattc aaagacattg caagcacgtt gaacaaggcc 900

aaaagcatcg ttggtactac cgcgtcgttg cagtatatga agaatgtgtt taaagagaag 960

tacctgctgt ccgaggatac ctccggcaag tttagcgttg ataagctgaa gtttgacaaa 1020

ctgtacaaga tgctgaccga gatttacacc gaggacaact ttgtgaaatt cttcaaagtg 1080

ttgaatcgta aaacctatct gaattttgac aaagcggttt tcaagattaa catcgtgccg 1140

aaggtgaact acaccatcta tgacggtttt aacctgcgta acaccaacct ggcggcgaac 1200

tttaacggtc agaatacgga aatcaacaac atgaatttca cgaagttgaa gaacttcacg 1260

ggtctgttcg agttctataa gctgctgtgc gtgcgcggta tcatcaccag caaaaccaaa 1320

agcctggaca aaggctacaa caaggcgctg aatgacctgt gcattaaggt aaacaattgg 1380

gatctgttct tttcgccatc cgaagataat tttaccaacg acctgaacaa gggtgaagaa 1440

atcaccagcg atacgaatat tgaagcagcg gaagagaata tcagcctgga tctgatccag 1500

cagtactatc tgacctttaa cttcgacaat gaaccggaga acattagcat tgagaatctg 1560

agcagcgaca ttatcggtca gctggaactg atgccgaata tcgaacgttt cccgaacggc 1620

aaaaagtacg agctggacaa gtacactatg ttccattacc tgcgtgcaca ggagtttgaa 1680

cacggtaaaa gccgtatcgc gctgaccaac agcgttaacg aggccctgct gaacccgagc 1740

cgtgtctata ccttcttcag cagcgactat gttaagaaag tgaacaaagc cactgaggcc 1800

gcgatgttcc tgggctgggt ggaacagctg gtatatgact tcacggacga gacgagcgaa 1860

gtgagcacta ccgacaaaat tgctgatatt accatcatta tcccgtatat tggtccggca 1920

ctgaacattg gcaacatgct gtacaaagac gattttgtgg gtgccctgat cttctccggt 1980

gccgtgattc tgctggagtt cattccggag attgcgatcc cggtgttggg taccttcgcg 2040

ctggtgtcct acatcgcgaa taaggttctg acggttcaga ccatcgataa cgcgctgtcg 2100

aaacgtaatg aaaaatggga cgaggtttac aaatacattg ttacgaattg gctggcgaaa 2160

gtcaataccc agatcgacct gatccgtaag aaaatgaaag aggcgctgga gaatcaggcg 2220

gaggccacca aagcaattat caactaccaa tacaaccagt acacggaaga agagaagaat 2280

aacattaact tcaatatcga tgatttgagc agcaagctga atgaatctat caacaaagcg 2340

atgatcaata tcaacaagtt tttgaatcag tgtagcgttt cgtacctgat gaatagcatg 2400

attccgtatg gcgtcaaacg tctggaggac ttcgacgcca gcctgaaaga tgcgttgctg 2460

aaatacattt acgacaatcg tggtacgctg attggccaag ttgaccgctt gaaagacaaa 2520

gttaacaata ccctgagcac cgacatccca tttcaactga gcaagtatgt tgataatcaa 2580

cgtctgttga gcactttcac cgagtatatc aaaaacatca tcaatactag cattctgaac 2640

ctgcgttacg agagcaagca tctgattgat ctgagccgtt atgctagcaa gatcaacatc 2700

ggtagcaagg tcaattttga cccgatcgat aagaaccaga tccagctgtt taatctggaa 2760

tcgagcaaaa ttgaggttat cctgaaaaag gccattgtct acaactccat gtacgagaat 2820

ttctccacca gcttctggat tcgcatcccg aaatacttca acaagattag cctgaacaac 2880

gagtatacta tcatcaactg tatggagaac aacagcggtt ggaaggtgtc tctgaactat 2940

ggtgagatca tttggacctt gcaggacacc aaagagatca agcagcgcgt cgtgttcaag 3000

tactctcaaa tgatcaacat ttccgattac attaatcgtt ggatcttcgt gaccattacg 3060

aataaccgtc tgaataagag caagatttac atcaatggtc gcttgatcga tcagaaaccg 3120

attagcaacc tgggtaatat ccacgcaagc aacaagatta tgttcaaatt ggacggttgc 3180

cgcgataccc atcgttatat ctggatcaag tatttcaacc tgtttgataa agaactgaat 3240

gagaaggaga tcaaagattt gtatgacaac caatctaaca gcggcatttt gaaggacttc 3300

tggggcgatt atctgcaata cgataagccg tactatatgc tgaacctgta tgatccgaac 3360

aaatatgtgg atgtcaataa tgtgggtatt cgtggttaca tgtatttgaa gggtccgcgt 3420

ggcagcgtta tgacgaccaa catttacctg aactctagcc tgtaccgtgg tacgaaattc 3480

atcattaaga aatatgccag cggcaacaaa gataacattg tgcgtaataa cgatcgtgtc 3540

tacatcaacg tggtcgtgaa gaataaagag taccgtctgg cgaccaacgc ttcgcaggcg 3600

ggtgttgaga aaattctgag cgcgttggag atccctgatg tcggtaatct gagccaagtc 3660

gtggttatga agagcaagaa cgacaagggt atcactaaca agtgcaagat gaacctgcaa 3720

gacaacaatg gtaacgacat cggctttatt ggtttccacc agttcaacaa tattgctaaa 3780

ctggtagcga gcaattggta caatcgtcag attgagcgca gcagccgtac tttgggctgt 3840

agctgggagt ttatcccggt cgatgatggt tggggcgaac gtccgctg 3888

<210> 4
<211> 1296
<212> PRT
<213> Artificial Sequence

<220>
<223> Engineered BoNT/A1

<400> 4

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15

Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30

Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
85 90 95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110

Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
130 135 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145 150 155 160

```
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
                165                 170                 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
            180                 185                 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
        195                 200                 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
    210                 215                 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225                 230                 235                 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
                245                 250                 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
            260                 265                 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
        275                 280                 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
    290                 295                 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305                 310                 315                 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
                325                 330                 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
            340                 345                 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
        355                 360                 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
    370                 375                 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385                 390                 395                 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
                405                 410                 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
            420                 425                 430
```

```
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys
        435                 440                 445

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
    450                 455                 460

Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465                 470                 475                 480

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
                485                 490                 495

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
            500                 505                 510

Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
        515                 520                 525

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
    530                 535                 540

Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
545                 550                 555                 560

His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
                565                 570                 575

Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
            580                 585                 590

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
        595                 600                 605

Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
    610                 615                 620

Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
625                 630                 635                 640

Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
                645                 650                 655

Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
            660                 665                 670

Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
        675                 680                 685

Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
    690                 695                 700
```

```
Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705                 710                 715                 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
                725                 730                 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
            740                 745                 750

Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
        755                 760                 765

Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
    770                 775                 780

Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785                 790                 795                 800

Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
                805                 810                 815

Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
            820                 825                 830

Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
        835                 840                 845

Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
    850                 855                 860

Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865                 870                 875                 880

Leu Arg Tyr Glu Ser Lys His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
                885                 890                 895

Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
            900                 905                 910

Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
        915                 920                 925

Lys Lys Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
    930                 935                 940

Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Lys Ile Ser Leu Asn Asn
945                 950                 955                 960

Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
                965                 970                 975
```

```
Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Lys Glu
            980                 985                 990

Ile Lys Gln Arg Val Val Phe Lys  Tyr Ser Gln Met Ile  Asn Ile Ser
        995                 1000                1005

Asp Tyr  Ile Asn Arg Trp Ile  Phe Val Thr Ile Thr  Asn Asn Arg
    1010                1015                1020

Leu Asn  Lys Ser Lys Ile Tyr  Ile Asn Gly Arg Leu  Ile Asp Gln
    1025                1030                1035

Lys Pro  Ile Ser Asn Leu Gly  Asn Ile His Ala Ser  Asn Lys Ile
    1040                1045                1050

Met Phe  Lys Leu Asp Gly Cys  Arg Asp Thr His Arg  Tyr Ile Trp
    1055                1060                1065

Ile Lys  Tyr Phe Asn Leu Phe  Asp Lys Glu Leu Asn  Glu Lys Glu
    1070                1075                1080

Ile Lys  Asp Leu Tyr Asp Asn  Gln Ser Asn Ser Gly  Ile Leu Lys
    1085                1090                1095

Asp Phe  Trp Gly Asp Tyr Leu  Gln Tyr Asp Lys Pro  Tyr Tyr Met
    1100                1105                1110

Leu Asn  Leu Tyr Asp Pro Asn  Lys Tyr Val Asp Val  Asn Asn Val
    1115                1120                1125

Gly Ile  Arg Gly Tyr Met Tyr  Leu Lys Gly Pro Arg  Gly Ser Val
    1130                1135                1140

Met Thr  Thr Asn Ile Tyr Leu  Asn Ser Ser Leu Tyr  Arg Gly Thr
    1145                1150                1155

Lys Phe  Ile Ile Lys Lys Tyr  Ala Ser Gly Asn Lys  Asp Asn Ile
    1160                1165                1170

Val Arg  Asn Asn Asp Arg Val  Tyr Ile Asn Val Val  Val Lys Asn
    1175                1180                1185

Lys Glu  Tyr Arg Leu Ala Thr  Asn Ala Ser Gln Ala  Gly Val Glu
    1190                1195                1200

Lys Ile  Leu Ser Ala Leu Glu  Ile Pro Asp Val Gly  Asn Leu Ser
    1205                1210                1215

Gln Val  Val Val Met Lys Ser  Lys Asn Asp Lys Gly  Ile Thr Asn
    1220                1225                1230
```

Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly
1235 1240 1245

Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala
1250 1255 1260

Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu
1265 1270 1275

Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu
1280 1285 1290

Arg Pro Leu
1295

<210> 5
<211> 3888
<212> DNA
<213> Artificial Sequence

<220>
<223> Engineered BoNT/A1

<400> 5
atgccattcg tcaacaagca attcaactac aaagacccag tcaacggcgt cgacatcgca 60

tacatcaaga ttccgaacgc cggtcaaatg cagccggtta aggcttttaa gatccacaac 120

aagatttggg ttatcccgga gcgtgacacc ttcacgaacc cggaagaagg cgatctgaac 180

ccgccaccgg aagcgaagca agtccctgtc agctactacg attcgacgta cctgagcacg 240

gataacgaaa aagataacta cctgaaaggt gtgaccaagc tgttcgaacg tatctacagc 300

acggatctgg gtcgcatgct gctgactagc attgttcgcg gtatcccgtt ctggggtggt 360

agcacgattg acaccgaact gaaggttatc gacactaact gcattaacgt tattcaaccg 420

gatggtagct atcgtagcga agagctgaat ctggtcatca ttggcccgag cgcagacatt 480

atccaattcg agtgcaagag ctttggtcac gaggttctga atctgacccg caatggctat 540

ggtagcaccc agtacattcg tttttcgccg gattttacct tcggctttga agagagcctg 600

gaggttgata ccaatccgtt gctgggtgcg ggcaaattcg ctaccgatcc ggctgtcacg 660

ctggcccatg aactgatcca cgcaggccac cgcctgtacg gcattgccat caacccaaac 720

cgtgtgttca aggttaatac gaatgcatac tacgagatga gcggcctgga agtcagcttc 780

gaagaactgc gcaccttcgg tggccatgac gctaaattca ttgacagctt gcaagagaat 840

gagttccgtc tgtactacta taacaaattc aaagacattg caagcacgtt gaacaaggcc 900

aaaagcatcg ttggtactac cgcgtcgttg cagtatatga agaatgtgtt taaagagaag 960

tacctgctgt ccgaggatac ctccggcaag tttagcgttg ataagctgaa gtttgacaaa 1020

ctgtacaaga tgctgaccga gatttacacc gaggacaact ttgtgaaatt cttcaaagtg 1080

ttgaatcgta aaacctatct gaattttgac aaagcggttt tcaagattaa catcgtgccg 1140

aaggtgaact acaccatcta tgacggtttt aacctgcgta acaccaacct ggcggcgaac 1200

tttaacggtc agaatacgga aatcaacaac atgaatttca cgaagttgaa gaacttcacg 1260

ggtctgttcg agttctataa gctgctgtgc gtgcgcggta tcatcaccag caaaaccaaa 1320

agcctggaca aaggctacaa caaggcgctg aatgacctgt gcattaaggt aaacaattgg 1380

gatctgttct tttcgccatc cgaagataat tttaccaacg acctgaacaa gggtgaagaa 1440

atcaccagcg atacgaatat tgaagcagcg gaagagaata tcagcctgga tctgatccag 1500

cagtactatc tgacctttaa cttcgacaat gaaccggaga acattagcat tgagaatctg 1560

agcagcgaca ttatcggtca gctggaactg atgccgaata tcgaacgttt cccgaacggc 1620

aaaaagtacg agctggacaa gtacactatg ttccattacc tgcgtgcaca ggagtttgaa 1680

cacggtaaaa gccgtatcgc gctgaccaac agcgttaacg aggccctgct gaacccgagc 1740

cgtgtctata ccttcttcag cagcgactat gttaagaaag tgaacaaagc cactgaggcc 1800

gcgatgttcc tgggctgggt ggaacagctg gtatatgact tcacggacga gacgagcgaa 1860

gtgagcacta ccgacaaaat tgctgatatt accatcatta tcccgtatat tggtccggca 1920

ctgaacattg gcaacatgct gtacaaagac gattttgtgg gtgccctgat cttctccggt 1980

gccgtgattc tgctggagtt cattccggag attgcgatcc cggtgttggg taccttcgcg 2040

ctggtgtcct acatcgcgaa taaggttctg acggttcaga ccatcgataa cgcgctgtcg 2100

aaacgtaatg aaaaatggga cgaggtttac aaatacattg ttacgaattg gctggcgaaa 2160

gtcaataccc agatcgacct gatccgtaag aaaatgaaag aggcgctgga gaatcaggcg 2220

gaggccacca aagcaattat caactaccaa tacaaccagt acacggaaga agagaagaat 2280

aacattaact tcaatatcga tgatttgagc agcaagctga atgaatctat caacaaagcg 2340

atgatcaata tcaacaagtt tttgaatcag tgtagcgttt cgtacctgat gaatagcatg 2400

attccgtatg gcgtcaaacg tctggaggac ttcgacgcca gcctgaaaga tgcgttgctg 2460

aaatacattt acgacaatcg tggtacgctg attggccaag ttgaccgctt gaaagacaaa 2520

gttaacaata ccctgagcac cgacatccca tttcaactga gcaagtatgt tgataatcaa 2580

cgtctgttga gcactttcac cgagtatatc aaaaacatca tcaatactag cattctgaac 2640

ctgcgttacg agagcaatca tctgattgat ctgagccgtt atgctagcaa gatcaacatc 2700

ggtagcaagg tcaattttga cccgatcgat aagaaccaga tccagctgtt taatctggaa 2760

tcgagcaaaa ttgaggttat cctgaaaaag gccattgtct acaactccat gtacgagaat 2820

ttctccacca gcttctggat tcgcatcccg aaatacttca agaagattag cctgaacaac 2880

gagtatacta tcatcaactg tatggagaac aacagcggtt ggaaggtgtc tctgaactat 2940

ggtgagatca tttggacctt gcaggacacc aaagagatca agcagcgcgt cgtgttcaag 3000

tactctcaaa tgatcaacat ttccgattac attaatcgtt ggatcttcgt gaccattacg 3060

aataaccgtc tgaataagag caagatttac atcaatggtc gcttgatcga tcagaaaccg 3120

attagcaacc tgggtaatat ccacgcaagc aacaagatta tgttcaaatt ggacggttgc 3180

cgcgataccc atcgttatat ctggatcaag tatttcaacc tgtttgataa agaactgaat 3240

gagaaggaga tcaaagattt gtatgacaac caatctaaca gcggcatttt gaaggacttc 3300

tggggcgatt atctgcaata cgataagccg tactatatgc tgaacctgta tgatccgaac 3360

aaatatgtgg atgtcaataa tgtgggtatt cgtggttaca tgtatttgaa gggtccgcgt 3420

ggcagcgtta tgacgaccaa catttacctg aactctagcc tgtaccgtgg tacgaaattc 3480

atcattaaga aatatgccag cggcaacaaa gataacattg tgcgtaataa cgatcgtgtc 3540

tacatcaacg tggtcgtgaa gaataaagag taccgtctgg cgaccaacgc ttcgcaggcg 3600

ggtgttgaga aaattctgag cgcgttggag atccctgatg tcggtaatct gagccaagtc 3660

gtggttatga agagcaagaa cgacaagggt atcactaaca agtgcaagat gaacctgcaa 3720

gacaacaatg gtaacgacat cggctttatt ggtttccacc agttcaacaa tattgctaaa 3780

ctggtagcga gcaattggta caatcgtcag attgagcgca gcagccgtac tttgggctgt 3840

agctgggagt ttatcccggt cgatgatggt tggggcgaac gtccgctg 3888

<210> 6
<211> 1296
<212> PRT
<213> Artificial Sequence

<220>
<223> Engineered BoNT/A1

<400> 6

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15

Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30

Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
85 90 95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110

Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr

130 135 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145 150 155 160

Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
165 170 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
180 185 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
195 200 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
210 215 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225 230 235 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
245 250 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
260 265 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
275 280 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
290 295 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
325 330 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
355 360 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
370 375 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385 390 395 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu

405 410 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
420 425 430

Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys
435 440 445

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
450 455 460

Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465 470 475 480

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
485 490 495

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
500 505 510

Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
515 520 525

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
530 535 540

Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
545 550 555 560

His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
565 570 575

Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
580 585 590

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
595 600 605

Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
610 615 620

Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
625 630 635 640

Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
645 650 655

Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
660 665 670

Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys

675 680 685

Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
690 695 700

Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705 710 715 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
725 730 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
740 745 750

Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
755 760 765

Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
770 775 780

Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785 790 795 800

Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
805 810 815

Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
820 825 830

Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
835 840 845

Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
850 855 860

Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865 870 875 880

Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
885 890 895

Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
900 905 910

Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
915 920 925

Lys Lys Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
930 935 940

Phe Trp Ile Arg Ile Pro Lys Tyr Phe Lys Lys Ile Ser Leu Asn Asn

945 950 955 960

Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
965 970 975

Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Lys Glu
980 985 990

Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser
995 1000 1005

Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg
1010 1015 1020

Leu Asn Lys Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln
1025 1030 1035

Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Lys Ile
1040 1045 1050

Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
1055 1060 1065

Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu
1070 1075 1080

Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys
1085 1090 1095

Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met
1100 1105 1110

Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val
1115 1120 1125

Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val
1130 1135 1140

Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
1145 1150 1155

Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile
1160 1165 1170

Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn
1175 1180 1185

Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu
1190 1195 1200

Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser

1205 1210 1215

Gln Val Val Val Met Lys Ser Lys Asn Asp Lys Gly Ile Thr Asn
1220 1225 1230

Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly
1235 1240 1245

Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala
1250 1255 1260

Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu
1265 1270 1275

Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu
1280 1285 1290

Arg Pro Leu
1295

<210> 7
<211> 3888
<212> DNA
<213> Artificial Sequence

<220>
<223> Engineered BoNT/A1

<400> 7
atgccattcg tcaacaagca attcaactac aaagacccag tcaacggcgt cgacatcgca 60

tacatcaaga ttccgaacgc cggtcaaatg cagccggtta aggcttttaa gatccacaac 120

aagatttggg ttatcccgga gcgtgacacc ttcacgaacc cggaagaagg cgatctgaac 180

ccgccaccgg aagcgaagca agtccctgtc agctactacg attcgacgta cctgagcacg 240

gataacgaaa aagataacta cctgaaaggt gtgaccaagc tgttcgaacg tatctacagc 300

acggatctgg gtcgcatgct gctgactagc attgttcgcg gtatcccgtt ctggggtggt 360

agcacgattg acaccgaact gaaggttatc gacactaact gcattaacgt tattcaaccg 420

gatggtagct atcgtagcga agagctgaat ctggtcatca ttggcccgag cgcagacatt 480

atccaattcg agtgcaagag ctttggtcac gaggttctga atctgacccg caatggctat 540

ggtagcaccc agtacattcg tttttcgccg gattttacct tcggctttga agagagcctg 600

gaggttgata ccaatccgtt gctgggtgcg ggcaaattcg ctaccgatcc ggctgtcacg 660

ctggcccatg aactgatcca cgcaggccac cgcctgtacg gcattgccat caacccaaac 720

cgtgtgttca aggttaatac gaatgcatac tacgagatga gcggcctgga agtcagcttc 780

gaagaactgc gcaccttcgg tggccatgac gctaaattca ttgacagctt gcaagagaat 840

gagttccgtc tgtactacta taacaaattc aaagacattg caagcacgtt gaacaaggcc 900

aaaagcatcg ttggtactac cgcgtcgttg cagtatatga agaatgtgtt taaagagaag 960

tacctgctgt ccgaggatac ctccggcaag tttagcgttg ataagctgaa gtttgacaaa 1020

ctgtacaaga tgctgaccga gatttacacc gaggacaact ttgtgaaatt cttcaaagtg 1080

ttgaatcgta aaacctatct gaattttgac aaagcggttt tcaagattaa catcgtgccg 1140

aaggtgaact acaccatcta tgacggtttt aacctgcgta acaccaacct ggcggcgaac 1200

tttaacggtc agaatacgga aatcaacaac atgaatttca cgaagttgaa gaacttcacg 1260

ggtctgttcg agttctataa gctgctgtgc gtgcgcggta tcatcaccag caaaaccaaa 1320

agcctggaca aaggctacaa caaggcgctg aatgacctgt gcattaaggt aaacaattgg 1380

gatctgttct tttcgccatc cgaagataat tttaccaacg acctgaacaa gggtgaagaa 1440

atcaccagcg atacgaatat tgaagcagcg gaagagaata tcagcctgga tctgatccag 1500

cagtactatc tgacctttaa cttcgacaat gaaccggaga acattagcat tgagaatctg 1560

agcagcgaca ttatcggtca gctggaactg atgccgaata tcgaacgttt cccgaacggc 1620

aaaaagtacg agctggacaa gtacactatg ttccattacc tgcgtgcaca ggagtttgaa 1680

cacggtaaaa gccgtatcgc gctgaccaac agcgttaacg aggccctgct gaacccgagc 1740

cgtgtctata ccttcttcag cagcgactat gttaagaaag tgaacaaagc cactgaggcc 1800

gcgatgttcc tgggctgggt ggaacagctg gtatatgact tcacggacga gacgagcgaa 1860

gtgagcacta ccgacaaaat tgctgatatt accatcatta tcccgtatat tggtccggca 1920

ctgaacattg gcaacatgct gtacaaagac gattttgtgg gtgccctgat cttctccggt 1980

gccgtgattc tgctggagtt cattccggag attgcgatcc cggtgttggg taccttcgcg 2040

ctggtgtcct acatcgcgaa taaggttctg acggttcaga ccatcgataa cgcgctgtcg 2100

aaacgtaatg aaaaatggga cgaggtttac aaatacattg ttacgaattg gctggcgaaa 2160

gtcaataccc agatcgacct gatccgtaag aaaatgaaag aggcgctgga gaatcaggcg 2220

gaggccacca aagcaattat caactaccaa tacaaccagt acacggaaga agagaagaat 2280

aacattaact tcaatatcga tgatttgagc agcaagctga atgaatctat caacaaagcg 2340

atgatcaata tcaacaagtt tttgaatcag tgtagcgttt cgtacctgat gaatagcatg 2400

attccgtatg gcgtcaaacg tctggaggac ttcgacgcca gcctgaaaga tgcgttgctg 2460

aaatacattt acgacaatcg tggtacgctg attggccaag ttgaccgctt gaaagacaaa 2520

gttaacaata ccctgagcac cgacatccca tttcaactga gcaagtatgt tgataatcaa 2580

cgtctgttga gcactttcac cgagtatatc aaaaacatca tcaatactag cattctgaac 2640

ctgcgttacg agagcaatca tctgattgat ctgagccgtt atgctagcaa gatcaacatc 2700

ggtagcaagg tcaattttga cccgatcgat aagaaccaga tccagctgtt taatctggaa 2760

tcgagcaaaa ttgaggttat cctgaaaaag gccattgtct acaactccat gtacgagaat 2820

ttctccacca gcttctggat tcgcatcccg aaatacttca acaagattag cctgaacaac 2880

gagtatacta tcatcaactg tatggagaac aacagcggtt ggaaggtgtc tctgaactat 2940

ggtgagatca tttggacctt gcaggacacc aaagagatca agcagcgcgt cgtgttcaag 3000

tactctcaaa tgatcaacat ttccgattac attaatcgtt ggatcttcgt gaccattacg 3060

aataaccgtc tgaagaagag caagatttac atcaatggtc gcttgatcga tcagaaaccg 3120

attagcaacc tgggtaatat ccacgcaagc aacaagatta tgttcaaatt ggacggttgc 3180

cgcgataccc atcgttatat ctggatcaag tatttcaacc tgtttgataa agaactgaat 3240

gagaaggaga tcaaagattt gtatgacaac caatctaaca gcggcatttt gaaggacttc 3300

tggggcgatt atctgcaata cgataagccg tactatatgc tgaacctgta tgatccgaac 3360

aaatatgtgg atgtcaataa tgtgggtatt cgtggttaca tgtatttgaa gggtccgcgt 3420

ggcagcgtta tgacgaccaa catttacctg aactctagcc tgtaccgtgg tacgaaattc 3480

atcattaaga aatatgccag cggcaacaaa gataacattg tgcgtaataa cgatcgtgtc 3540

tacatcaacg tggtcgtgaa gaataaagag taccgtctgg cgaccaacgc ttcgcaggcg 3600

ggtgttgaga aaattctgag cgcgttggag atccctgatg tcggtaatct gagccaagtc 3660

gtggttatga agagcaagaa cgacaagggt atcactaaca agtgcaagat gaacctgcaa 3720

gacaacaatg gtaacgacat cggctttatt ggtttccacc agttcaacaa tattgctaaa 3780

ctggtagcga gcaattggta caatcgtcag attgagcgca gcagccgtac tttgggctgt 3840

agctgggagt ttatcccggt cgatgatggt tggggcgaac gtccgctg 3888

<210> 8
<211> 1296
<212> PRT
<213> Artificial Sequence

<220>
<223> Engineered BoNT/A1

<400> 8

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15

Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30

Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
85 90 95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110

```
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
        115                 120                 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
    130                 135                 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145                 150                 155                 160

Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
                165                 170                 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
            180                 185                 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
        195                 200                 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
    210                 215                 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225                 230                 235                 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
                245                 250                 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
            260                 265                 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
        275                 280                 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
    290                 295                 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305                 310                 315                 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
                325                 330                 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
            340                 345                 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
        355                 360                 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
    370                 375                 380
```

```
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385                 390                 395                 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
                405                 410                 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
            420                 425                 430

Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys
        435                 440                 445

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
    450                 455                 460

Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465                 470                 475                 480

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
                485                 490                 495

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
            500                 505                 510

Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
        515                 520                 525

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
    530                 535                 540

Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
545                 550                 555                 560

His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
                565                 570                 575

Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
            580                 585                 590

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
        595                 600                 605

Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
    610                 615                 620

Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
625                 630                 635                 640

Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
                645                 650                 655
```

```
Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
            660                 665                 670

Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
        675                 680                 685

Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
    690                 695                 700

Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705                 710                 715                 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
                725                 730                 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
            740                 745                 750

Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
        755                 760                 765

Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
    770                 775                 780

Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785                 790                 795                 800

Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
                805                 810                 815

Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
            820                 825                 830

Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
        835                 840                 845

Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
    850                 855                 860

Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865                 870                 875                 880

Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
                885                 890                 895

Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
            900                 905                 910

Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
        915                 920                 925
```

```
Lys Lys Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
    930                 935                 940

Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Lys Ile Ser Leu Asn Asn
945                 950                 955                 960

Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
                965                 970                 975

Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Lys Glu
            980                 985                 990

Ile Lys Gln Arg Val Val Phe Lys  Tyr Ser Gln Met Ile  Asn Ile Ser
        995                 1000                1005

Asp Tyr  Ile Asn Arg Trp Ile  Phe Val Thr Ile Thr  Asn Asn Arg
    1010                1015                1020

Leu Lys  Lys Ser Lys Ile Tyr  Ile Asn Gly Arg Leu  Ile Asp Gln
    1025                1030                1035

Lys Pro  Ile Ser Asn Leu Gly  Asn Ile His Ala Ser  Asn Lys Ile
    1040                1045                1050

Met Phe  Lys Leu Asp Gly Cys  Arg Asp Thr His Arg  Tyr Ile Trp
    1055                1060                1065

Ile Lys  Tyr Phe Asn Leu Phe  Asp Lys Glu Leu Asn  Glu Lys Glu
    1070                1075                1080

Ile Lys  Asp Leu Tyr Asp Asn  Gln Ser Asn Ser Gly  Ile Leu Lys
    1085                1090                1095

Asp Phe  Trp Gly Asp Tyr Leu  Gln Tyr Asp Lys Pro  Tyr Tyr Met
    1100                1105                1110

Leu Asn  Leu Tyr Asp Pro Asn  Lys Tyr Val Asp Val  Asn Asn Val
    1115                1120                1125

Gly Ile  Arg Gly Tyr Met Tyr  Leu Lys Gly Pro Arg  Gly Ser Val
    1130                1135                1140

Met Thr  Thr Asn Ile Tyr Leu  Asn Ser Ser Leu Tyr  Arg Gly Thr
    1145                1150                1155

Lys Phe  Ile Ile Lys Lys Tyr  Ala Ser Gly Asn Lys  Asp Asn Ile
    1160                1165                1170

Val Arg  Asn Asn Asp Arg Val  Tyr Ile Asn Val Val  Val Lys Asn
    1175                1180                1185
```

```
Lys Glu  Tyr Arg Leu Ala Thr  Asn Ala Ser Gln Ala  Gly Val Glu
    1190                 1195                 1200

Lys Ile  Leu Ser Ala Leu Glu  Ile Pro Asp Val Gly  Asn Leu Ser
    1205                 1210                 1215

Gln Val  Val Val Met Lys Ser  Lys Asn Asp Lys Gly  Ile Thr Asn
    1220                 1225                 1230

Lys Cys  Lys Met Asn Leu Gln  Asp Asn Asn Gly Asn  Asp Ile Gly
    1235                 1240                 1245

Phe Ile  Gly Phe His Gln Phe  Asn Asn Ile Ala Lys  Leu Val Ala
    1250                 1255                 1260

Ser Asn  Trp Tyr Asn Arg Gln  Ile Glu Arg Ser Ser  Arg Thr Leu
    1265                 1270                 1275

Gly Cys  Ser Trp Glu Phe Ile  Pro Val Asp Asp Gly  Trp Gly Glu
    1280                 1285                 1290

Arg Pro  Leu
    1295
```

<210> 9
<211> 3888
<212> DNA
<213> Artificial Sequence

<220>
<223> Engineered BoNT/A1

<400> 9

```
atgccattcg tcaacaagca attcaactac aaagacccag tcaacggcgt cgacatcgca       60

tacatcaaga ttccgaacgc cggtcaaatg cagccggtta aggcttttaa gatccacaac      120

aagatttggg ttatcccgga gcgtgacacc ttcacgaacc cggaagaagg cgatctgaac      180

ccgccaccgg aagcgaagca agtccctgtc agctactacg attcgacgta cctgagcacg      240

gataacgaaa aagataacta cctgaaaggt gtgaccaagc tgttcgaacg tatctacagc      300

acggatctgg gtcgcatgct gctgactagc attgttcgcg gtatcccgtt ctggggtggt      360

agcacgattg acaccgaact gaaggttatc gacactaact gcattaacgt tattcaaccg      420

gatggtagct atcgtagcga agagctgaat ctggtcatca ttggcccgag cgcagacatt      480

atccaattcg agtgcaagag ctttggtcac gaggttctga atctgacccg caatggctat      540

ggtagcaccc agtacattcg tttttcgccg gattttacct tcggctttga agagagcctg      600

gaggttgata ccaatccgtt gctgggtgcg ggcaaattcg ctaccgatcc ggctgtcacg      660

ctggcccatg aactgatcca cgcaggccac cgcctgtacg gcattgccat caacccaaac      720

cgtgtgttca aggttaatac gaatgcatac tacgagatga gcggcctgga agtcagcttc      780
```

gaagaactgc gcaccttcgg tggccatgac gctaaattca ttgacagctt gcaagagaat 840

gagttccgtc tgtactacta taacaaattc aaagacattg caagcacgtt gaacaaggcc 900

aaaagcatcg ttggtactac cgcgtcgttg cagtatatga agaatgtgtt taaagagaag 960

tacctgctgt ccgaggatac ctccggcaag tttagcgttg ataagctgaa gtttgacaaa 1020

ctgtacaaga tgctgaccga gatttacacc gaggacaact ttgtgaaatt cttcaaagtg 1080

ttgaatcgta aaacctatct gaattttgac aaagcggttt tcaagattaa catcgtgccg 1140

aaggtgaact acaccatcta tgacggtttt aacctgcgta acaccaacct ggcggcgaac 1200

tttaacggtc agaatacgga aatcaacaac atgaatttca cgaagttgaa gaacttcacg 1260

ggtctgttcg agttctataa gctgctgtgc gtgcgcggta tcatcaccag caaaaccaaa 1320

agcctggaca aaggctacaa caaggcgctg aatgacctgt gcattaaggt aaacaattgg 1380

gatctgttct tttcgccatc cgaagataat tttaccaacg acctgaacaa gggtgaagaa 1440

atcaccagcg atacgaatat tgaagcagcg gaagagaata tcagcctgga tctgatccag 1500

cagtactatc tgacctttaa cttcgacaat gaaccggaga acattagcat tgagaatctg 1560

agcagcgaca ttatcggtca gctggaactg atgccgaata tcgaacgttt cccgaacggc 1620

aaaaagtacg agctggacaa gtacactatg ttccattacc tgcgtgcaca ggagtttgaa 1680

cacggtaaaa gccgtatcgc gctgaccaac agcgttaacg aggccctgct gaacccgagc 1740

cgtgtctata ccttcttcag cagcgactat gttaagaaag tgaacaaagc cactgaggcc 1800

gcgatgttcc tgggctgggt ggaacagctg gtatatgact tcacggacga gacgagcgaa 1860

gtgagcacta ccgacaaaat tgctgatatt accatcatta tcccgtatat tggtccggca 1920

ctgaacattg gcaacatgct gtacaaagac gattttgtgg gtgccctgat cttctccggt 1980

gccgtgattc tgctggagtt cattccggag attgcgatcc cggtgttggg taccttcgcg 2040

ctggtgtcct acatcgcgaa taaggttctg acggttcaga ccatcgataa cgcgctgtcg 2100

aaacgtaatg aaaaatggga cgaggtttac aaatacattg ttacgaattg gctggcgaaa 2160

gtcaataccc agatcgacct gatccgtaag aaaatgaaag aggcgctgga gaatcaggcg 2220

gaggccacca aagcaattat caactaccaa tacaaccagt acacggaaga agagaagaat 2280

aacattaact tcaatatcga tgatttgagc agcaagctga atgaatctat caacaaagcg 2340

atgatcaata tcaacaagtt tttgaatcag tgtagcgttt cgtacctgat gaatagcatg 2400

attccgtatg gcgtcaaacg tctggaggac ttcgacgcca gcctgaaaga tgcgttgctg 2460

aaatacattt acgacaatcg tggtacgctg attggccaag ttgaccgctt gaaagacaaa 2520

gttaacaata ccctgagcac cgacatccca tttcaactga gcaagtatgt tgataatcaa 2580

cgtctgttga gcactttcac cgagtatatc aaaaacatca tcaatactag cattctgaac 2640

ctgcgttacg agagcaatca tctgattgat ctgagccgtt atgcaagcaa gatcaacatc 2700

ggtagcaagg tcaattttga cccgatcgat aagaaccaga tccagctgtt taatctggaa 2760

tcgagcaaaa ttgaggttat cctgaaaaac gccattgtct acaactccat gtacgagaat 2820

ttctccacca gcttctggat tcgcatcccg aaatacttca acagcattag cctgaacaac 2880

gagtatacta tcatcaactg tatggagaac aacagcggtt ggaaggtgtc tctgaactat 2940

ggtgagatca tttggacctt gcaggacacc caagagatca agcagcgcgt cgtgttcaag 3000

tactctcaaa tgatcaacat ttccgattac attaatcgtt ggatcttcgt gaccattacg 3060

aataaccgtc tgaataacag caagatttac atcaatggtc gcttgatcga tcagaaaccg 3120

attagcaacc tgggtaatat ccacgcaagc aacaacatta tgttcaaatt ggacggttgc 3180

cgcgataccc atcgttatat ctggatcaag tatttcaacc tgtttgataa agaactgaat 3240

gagaaggaga tcaaagattt gtatgacaac caatctaaca gcggcatttt gaaggacttc 3300

tggggcgatt atctgcaata cgataagccg tactatatgc tgaacctgta tgatccgaac 3360

aaatatgtgg atgtcaataa tgtgggtatt cgtggttaca tgtatttgaa gggtccgcgt 3420

ggcagcgtta tgacgaccaa catttacctg aactctagcc tgtaccgtgg tacgaaattc 3480

atcattaaga aatatgccag cggcaacaaa gataacattg tgcgtaataa cgatcgtgtc 3540

tacatcaacg tggtcgtgaa gcgtaaagag taccgtctgg cgaccaacgc ttcgcaggcg 3600

ggtgttgaga aaattctgag cgcgttggag atccctcgtg tccgtcgtct gagccaagtc 3660

gtggttatga agagcaagaa cgaccagggt atcactaaca agtgcaagat gaacctgcaa 3720

gaccgtcgtg gtaacgacat cggctttatt ggtttccacc agttcaacaa tattgctaaa 3780

ctggtagcga gcaattggta caatcgtcag attgagcgcc gtagccgtcg tttgggctgt 3840

agctgggagt ttatcccggt cgatgatggt tggggcgaac gtccgctg 3888

<210> 10
<211> 1296
<212> PRT
<213> Artificial Sequence

<220>
<223> Engineered BoNT/A1

<400> 10

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15

Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30

Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
35 40 45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
50 55 60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65 70 75 80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu

85 90 95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
100 105 110

Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
115 120 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
130 135 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145 150 155 160

Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
165 170 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
180 185 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
195 200 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
210 215 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225 230 235 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
245 250 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
260 265 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
275 280 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
290 295 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305 310 315 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
325 330 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
340 345 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn

```
        355                 360                 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
    370                 375                 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385                 390                 395                 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
                405                 410                 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
            420                 425                 430

Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys
        435                 440                 445

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
    450                 455                 460

Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465                 470                 475                 480

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
                485                 490                 495

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
            500                 505                 510

Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
        515                 520                 525

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
    530                 535                 540

Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
545                 550                 555                 560

His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
                565                 570                 575

Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
            580                 585                 590

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
        595                 600                 605

Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
    610                 615                 620

Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
```

625 630 635 640

Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
645 650 655

Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
660 665 670

Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
675 680 685

Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
690 695 700

Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705 710 715 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
725 730 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
740 745 750

Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
755 760 765

Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
770 775 780

Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785 790 795 800

Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
805 810 815

Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
820 825 830

Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
835 840 845

Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
850 855 860

Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865 870 875 880

Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
885 890 895

Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn

900 905 910

Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
915 920 925

Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
930 935 940

Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn
945 950 955 960

Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
965 970 975

Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu
980 985 990

Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser
995 1000 1005

Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg
1010 1015 1020

Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln
1025 1030 1035

Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile
1040 1045 1050

Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
1055 1060 1065

Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu
1070 1075 1080

Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys
1085 1090 1095

Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met
1100 1105 1110

Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val
1115 1120 1125

Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val
1130 1135 1140

Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
1145 1150 1155

Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile

```
    1160                1165                1170

Val Arg  Asn Asn Asp Arg Val  Tyr Ile Asn Val Val  Val Lys Arg
    1175                1180                1185

Lys Glu  Tyr Arg Leu Ala Thr  Asn Ala Ser Gln Ala  Gly Val Glu
    1190                1195                1200

Lys Ile  Leu Ser Ala Leu Glu  Ile Pro Arg Val Arg  Arg Leu Ser
    1205                1210                1215

Gln Val  Val Val Met Lys Ser  Lys Asn Asp Gln Gly  Ile Thr Asn
    1220                1225                1230

Lys Cys  Lys Met Asn Leu Gln  Asp Arg Arg Gly Asn  Asp Ile Gly
    1235                1240                1245

Phe Ile  Gly Phe His Gln Phe  Asn Asn Ile Ala Lys  Leu Val Ala
    1250                1255                1260

Ser Asn  Trp Tyr Asn Arg Gln  Ile Glu Arg Arg Ser  Arg Arg Leu
    1265                1270                1275

Gly Cys  Ser Trp Glu Phe Ile  Pro Val Asp Asp Gly  Trp Gly Glu
    1280                1285                1290

Arg Pro  Leu
    1295
```