eolf-seql 10
SEQUENCE LISTING

<110> Wageningen Universiteit & Stichting voor de Technische Wetenschappen

<120> THERMOSTABLE CAS9 NUCLEASES

<130> P220294WO3

<150> PCT/EP2016/081077

<151> 2016-12-14

<160> 170

<170> PatentIn version 3.5

<210> 1

<211> 1082

<212> PRT

<213> Geobacillus thermodenitrificans T12

<400> 1

Met Lys Tyr Lys Ile Gly Leu Asp Ile Gly Ile Thr Ser Ile Gly Trp

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Ala Val Ile Asn Leu Asp Ile Pro Arg Ile Glu Asp Leu Gly Val Arg
            20                  25                  30

Ile Phe Asp Arg Ala Glu Asn Pro Lys Thr Gly Glu Ser Leu Ala Leu
        35                  40                  45

Pro Arg Arg Leu Ala Arg Ser Ala Arg Arg Arg Leu Arg Arg Arg Lys
    50                  55                  60

His Arg Leu Glu Arg Ile Arg Arg Leu Phe Val Arg Glu Gly Ile Leu
65                  70                  75                  80

Thr Lys Glu Glu Leu Asn Lys Leu Phe Glu Lys Lys His Glu Ile Asp
                85                  90                  95

Val Trp Gln Leu Arg Val Glu Ala Leu Asp Arg Lys Leu Asn Asn Asp
            100                 105                 110

Glu Leu Ala Arg Ile Leu Leu His Leu Ala Lys Arg Arg Gly Phe Arg
        115                 120                 125
```

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Ser Asn Arg Lys Ser Glu Arg Thr Asn Lys Glu Asn Ser Thr Met Leu
    130                 135                 140

Lys His Ile Glu Glu Asn Gln Ser Ile Leu Ser Ser Tyr Arg Thr Val
145                 150                 155                 160

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

Lys Glu Asp Asn Tyr Thr Asn Thr Val Ala Arg Asp Asp Leu Glu Arg
            180                 185                 190

Glu Ile Lys Leu Ile Phe Ala Lys Gln Arg Glu Tyr Gly Asn Ile Val
        195                 200                 205

Cys Thr Glu Ala Phe Glu His Glu Tyr Ile Ser Ile Trp Ala Ser Gln
    210                 215                 220
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Arg Pro Phe Ala Ser Lys Asp Asp Ile Glu Lys Lys Val Gly Phe Cys
225                 230                 235                 240

Thr Phe Glu Pro Lys Glu Lys Arg Ala Pro Lys Ala Thr Tyr Thr Phe
                245                 250                 255

Gln Ser Phe Thr Val Trp Glu His Ile Asn Lys Leu Arg Leu Val Ser
            260                 265                 270

Pro Gly Gly Ile Arg Ala Leu Thr Asp Asp Glu Arg Arg Leu Ile Tyr
        275                 280                 285

Lys Gln Ala Phe His Lys Asn Lys Ile Thr Phe His Asp Val Arg Thr
    290                 295                 300

Leu Leu Asn Leu Pro Asp Asp Thr Arg Phe Lys Gly Leu Leu Tyr Asp
305                 310                 315                 320

Arg Asn Thr Thr Leu Lys Glu Asn Glu Lys Val Arg Phe Leu Glu Leu
                325                 330                 335
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Gly Ala Tyr His Lys Ile Arg Lys Ala Ile Asp Ser Val Tyr Gly Lys
340 345 350

Gly Ala Ala Lys Ser Phe Arg Pro Ile Asp Phe Asp Thr Phe Gly Tyr
355 360 365

Ala Leu Thr Met Phe Lys Asp Asp Thr Asp Ile Arg Ser Tyr Leu Arg
370 375 380

Asn Glu Tyr Glu Gln Asn Gly Lys Arg Met Glu Asn Leu Ala Asp Lys
385 390 395 400

Val Tyr Asp Glu Glu Leu Ile Glu Glu Leu Leu Asn Leu Ser Phe Ser
405 410 415

Lys Phe Gly His Leu Ser Leu Lys Ala Leu Arg Asn Ile Leu Pro Tyr
420 425 430

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Met Glu Gln Gly Glu Val Tyr Ser Thr Ala Cys Glu Arg Ala Gly Tyr
        435                 440                 445

Thr Phe Thr Gly Pro Lys Lys Lys Gln Lys Thr Val Leu Leu Pro Asn
    450                 455                 460

Ile Pro Pro Ile Ala Asn Pro Val Val Met Arg Ala Leu Thr Gln Ala
465                 470                 475                 480

Arg Lys Val Val Asn Ala Ile Ile Lys Lys Tyr Gly Ser Pro Val Ser
                485                 490                 495

Ile His Ile Glu Leu Ala Arg Glu Leu Ser Gln Ser Phe Asp Glu Arg
            500                 505                 510

Arg Lys Met Gln Lys Glu Gln Glu Gly Asn Arg Lys Lys Asn Glu Thr
        515                 520                 525

Ala Ile Arg Gln Leu Val Glu Tyr Gly Leu Thr Leu Asn Pro Thr Gly
    530                 535                 540
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Leu Asp Ile Val Lys Phe Lys Leu Trp Ser Glu Gln Asn Gly Lys Cys
545                 550                 555                 560

Ala Tyr Ser Leu Gln Pro Ile Glu Ile Glu Arg Leu Leu Glu Pro Gly
                565                 570                 575

Tyr Thr Glu Val Asp His Val Ile Pro Tyr Ser Arg Ser Leu Asp Asp
            580                 585                 590

Ser Tyr Thr Asn Lys Val Leu Val Leu Thr Lys Glu Asn Arg Glu Lys
        595                 600                 605

Gly Asn Arg Thr Pro Ala Glu Tyr Leu Gly Leu Gly Ser Glu Arg Trp
    610                 615                 620

Gln Gln Phe Glu Thr Phe Val Leu Thr Asn Lys Gln Phe Ser Lys Lys
625                 630                 635                 640
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Lys Arg Asp Arg Leu Leu Arg Leu His Tyr Asp Glu Asn Glu Glu Asn
                645                 650                 655

Glu Phe Lys Asn Arg Asn Leu Asn Asp Thr Arg Tyr Ile Ser Arg Phe
            660                 665                 670

Leu Ala Asn Phe Ile Arg Glu His Leu Lys Phe Ala Asp Ser Asp Asp
        675                 680                 685

Lys Gln Lys Val Tyr Thr Val Asn Gly Arg Ile Thr Ala His Leu Arg
    690                 695                 700

Ser Arg Trp Asn Phe Asn Lys Asn Arg Glu Glu Ser Asn Leu His His
705                 710                 715                 720

Ala Val Asp Ala Ala Ile Val Ala Cys Thr Thr Pro Ser Asp Ile Ala
                725                 730                 735

Arg Val Thr Ala Phe Tyr Gln Arg Arg Glu Gln Asn Lys Glu Leu Ser
            740                 745                 750
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Lys Lys Thr Asp Pro Gln Phe Pro Gln Pro Trp Pro His Phe Ala Asp
        755                 760                 765

Glu Leu Gln Ala Arg Leu Ser Lys Asn Pro Lys Glu Ser Ile Lys Ala
    770                 775                 780

Leu Asn Leu Gly Asn Tyr Asp Asn Glu Lys Leu Glu Ser Leu Gln Pro
785                 790                 795                 800

Val Phe Val Ser Arg Met Pro Lys Arg Ser Ile Thr Gly Ala Ala His
                805                 810                 815

Gln Glu Thr Leu Arg Arg Tyr Ile Gly Ile Asp Glu Arg Ser Gly Lys
            820                 825                 830

Ile Gln Thr Val Val Lys Lys Lys Leu Ser Glu Ile Gln Leu Asp Lys
        835                 840                 845
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Thr Gly His Phe Pro Met Tyr Gly Lys Glu Ser Asp Pro Arg Thr Tyr
850 855 860

Glu Ala Ile Arg Gln Arg Leu Leu Glu His Asn Asn Asp Pro Lys Lys
865 870 875 880

Ala Phe Gln Glu Pro Leu Tyr Lys Pro Lys Lys Asn Gly Glu Leu Gly
885 890 895

Pro Ile Ile Arg Thr Ile Lys Ile Ile Asp Thr Thr Asn Gln Val Ile
900 905 910

Pro Leu Asn Asp Gly Lys Thr Val Ala Tyr Asn Ser Asn Ile Val Arg
915 920 925

Val Asp Val Phe Glu Lys Asp Gly Lys Tyr Tyr Cys Val Pro Ile Tyr
930 935 940

Thr Ile Asp Met Met Lys Gly Ile Leu Pro Asn Lys Ala Ile Glu Pro
945 950 955 960

Asn Lys Pro Tyr Ser Glu Trp Lys Glu Met Thr Glu Asp Tyr Thr Phe
965 970 975

Arg Phe Ser Leu Tyr Pro Asn Asp Leu Ile Arg Ile Glu Phe Pro Arg
980 985 990

Glu Lys Thr Ile Lys Thr Ala Val Gly Glu Glu Ile Lys Ile Lys Asp
995 1000 1005

Leu Phe Ala Tyr Tyr Gln Thr Ile Asp Ser Ser Asn Gly Gly Leu
1010 1015 1020

Ser Leu Val Ser His Asp Asn Asn Phe Ser Leu Arg Ser Ile Gly
1025 1030 1035

Ser Arg Thr Leu Lys Arg Phe Glu Lys Tyr Gln Val Asp Val Leu
1040 1045 1050

Gly Asn Ile Tyr Lys Val Arg Gly Glu Lys Arg Val Gly Val Ala

1055 1060 1065

Ser Ser Ser His Ser Lys Ala Gly Glu Thr Ile Arg Pro Leu

1070 1075 1080

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<211> 8

<212> PRT

<213> Geobacillus thermodenitrificans T12

<400> 2

Glu Lys Asp Gly Lys Tyr Tyr Cys

1 5

<210> 3

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> amino acid motif from Cas9

<220>

<221> X

<222> (1)..(1)

<223> Isoleucine, Methionine or Proline

<220>

<221> X

<222> (2)..(2)

<223> Valine, Serine, Asparagine or Isoleucine

<220>

<221> X

<222> (5)..(5)

<223> Glutamate or Lysine

<220>

<221> X

<222> (6)..(6)

<223> Alanine, Glutamate or Arginine

<400> 3

Xaa Xaa Cys Thr Xaa Xaa

1 5

<210> 4

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> amino acid motif from G. thermodenitrificans T12 Cas9

<220>

<221> X

<222> (1)..(1)

<223> Methionine or Phenylalanine

<220>

<221> X

<222> (4)..(4)

<223> Histidine or Asparagine

<400> 4

Xaa Leu Lys Xaa Ile Glu

1 5

<210> 5

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> amino acid motif from G. thermodenitrificans T12

<220>

<221> X

<222> (1)..(1)

<223> Glutamate or Isoleucine

<220>

<221> X

<222> (5)..(5)

<223> Tryptophan, Serine or Lysine

<400> 5

Xaa Val Tyr Ser Xaa Lys

1 5

<210> 6

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> amino acid motif from thermophilic Cas9

<220>

<221> X

<222> (1)..(1)

<223> Alanine or Glutamate

<220>

<221> X

<222> (4)..(4)

<223> Glutamine or Lysine

<220>

<221> X

<222> (5)..(5)

<223> Arginine or Alanine

<220>

<221> X

<222> (9)..(9)

<223> Asparagine or Alanine

<220>

<221> X

<222> (12)..(12)

<223> Lysine or Serine

<400> 6

Xaa Phe Tyr Xaa Xaa Arg Glu Gln Xaa Lys Glu Xaa
1 5 10

<210> 7

<211> 3249

<212> DNA

<213> Geobacillus thermodenitrificans T12

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aaaaccgggg agtcactagc tcttccacgt cgcctcgccc gctccgcccg acgtcgtctg 180

cggcgtcgca aacatcgact ggagcgcatt cgccgcctgt tcgtccgcga aggaatttta 240

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ctggctaaac ggcgtggatt tagatccaac cgcaagagtg agcgcaccaa caaagaaaac 420

agtacgatgc tcaaacatat tgaagaaaac caatccattc tttcaagtta ccgaacggtt 480

gcagaaatgg ttgtcaagga tccgaaattt tccctgcaca agcgtaataa agaggataat 540

tacaccaaca ctgttgcccg cgacgatctt gaacgggaaa tcaaactgat tttcgccaaa 600

cagcgcgaat atgggaacat cgtttgcaca gaagcatttg aacacgagta tatttccatt 660

tgggcatcgc aacgcccttt tgcttctaag gatgatatcg agaaaaaagt cggtttctgt 720

acgtttgagc ctaaagaaaa acgcgcgcca aaagcaacat acacattcca gtccttcacc 780

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gatgatgaac gtcgtcttat atacaagcaa gcatttcata aaaataaaat caccttccat 900

gatgttcgaa cattgcttaa cttgcctgac gacacccgtt ttaaaggtct tttatatgac 960

cgaaacacca cgctgaagga aaatgagaaa gttcgcttcc ttgaactcgg cgcctatcat 1020

aaaatacgga aagcgatcga cagcgtctat ggcaaaggag cagcaaaatc atttcgtccg 1080

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ctatccctta aagcgcttcg caacatcctt ccatatatgg aacaaggcga agtctactca 1320

accgcttgtg aacgagcagg atatacattt acagggccaa agaaaaaaca gaaaacggta 1380

ttgctgccga acattccgcc gatcgccaat ccggtcgtca tgcgcgcact gacacaggca 1440

cgcaaagtgg tcaatgccat tatcaaaaag tacggctcac cggtctccat ccatatcgaa 1500

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ggaaaccgaa agaaaaacga aactgccatt cgccaacttg ttgaatatgg gctgacgctc 1620

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gcctattcac tccaaccgat cgaaatcgag cggttgctcg aaccaggcta tacagaagtc 1740

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tcagaacgtt ggcaacagtt cgagacgttt gtcttgacaa ataagcagtt ttcgaaaaag 1920

aagcgggatc gactccttcg gcttcattac gatgaaaacg aagaaaatga gtttaaaaat 1980

cgtaatctaa atgatacccg ttatatctca cgcttcttgg ctaactttat tcgcgaacat 2040

ctcaaattcg ccgacagcga tgacaaacaa aaagtataca cggtcaacgg ccgtattacc 2100

gcccatttac gcagccgttg gaattttaac aaaaaccggg aagaatcgaa tttgcatcat 2160

gccgtcgatg ctgccatcgt cgcctgcaca acgccgagcg atatcgcccg agtcaccgcc 2220

ttctatcaac ggcgcgaaca aaacaaagaa ctgtccaaaa agacggatcc gcagtttccg 2280

cagccttggc cgcactttgc tgatgaactg caggcgcgtt tatcaaaaaa tccaaaggag 2340

agtataaaag ctctcaatct tggaaattat gataacgaga aactcgaatc gttgcagccg 2400

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ggggaagaaa tcaaaattaa ggatctgttc gcctattatc aaaccatcga ctcctccaat 3060

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accctcaaac gattcgagaa ataccaagta gatgtgctag gcaacatcta caaagtgaga 3180

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ccgttataa 3249

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<211> 1045

<212> PRT

<213> Actinomyces naeslundii

<400> 8

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20 25 30

His Gly Thr Pro Ile Glu Leu Leu Ser Ala Leu Ser His Ile His Asp
35 40 45

Ser Gly Val Gly Lys Glu Gly Lys Lys Asp His Asp Thr Arg Lys Lys
50 55 60

Leu Ser Gly Ile Ala Arg Arg Ala Arg Arg Leu Leu His His Arg Arg
65 70 75 80

Thr Gln Leu Gln Gln Leu Asp Glu Val Leu Arg Asp Leu Gly Phe Pro
85 90 95

Ile Pro Thr Pro Gly Glu Phe Leu Asp Leu Asn Glu Gln Thr Asp Pro
100 105 110

Tyr Arg Val Trp Arg Val Arg Ala Arg Leu Val Glu Glu Lys Leu Pro
115 120 125

Glu Glu Leu Arg Gly Pro Ala Ile Ser Met Ala Val Arg His Ile Ala
130 135 140

Arg His Arg Gly Trp Arg Asn Pro Tyr Ser Lys Val Glu Ser Leu Leu
145 150 155 160

Ser Pro Ala Asn Ala Asn Glu Ile Arg Lys Ile Cys Ala Arg Gln Gly
165 170 175

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Val Ser Pro Asp Val Cys Lys Gln Leu Leu Arg Ala Val Phe Lys Ala
            180                 185                 190

Asp Ser Pro Arg Gly Ser Ala Val Ser Arg Val Ala Pro Asp Pro Leu
        195                 200                 205

Pro Gly Gln Gly Ser Phe Arg Arg Ala Pro Lys Cys Asp Pro Glu Phe
    210                 215                 220

Gln Arg Phe Arg Ile Ile Ser Ile Val Ala Asn Leu Arg Ile Ser Glu
225                 230                 235                 240

Thr Lys Gly Glu Asn Arg Pro Leu Thr Ala Asp Glu Arg Arg His Val
                245                 250                 255

Val Thr Phe Leu Thr Glu Asp Ser Gln Ala Asp Leu Thr Trp Val Asp
            260                 265                 270

Val Ala Glu Lys Leu Gly Val His Arg Arg Asp Leu Arg Gly Thr Ala
        275                 280                 285
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Val His Thr Asp Asp Gly Glu Arg Ser Ala Ala Arg Pro Pro Ile Asp
    290                 295                 300

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

Trp Trp Glu Glu Ala Asp Ser Glu Gln Arg Gly Ala Met Ile Arg Tyr
                325                 330                 335

Leu Tyr Glu Asp Pro Thr Asp Ser Glu Cys Ala Glu Ile Ile Ala Glu
            340                 345                 350

Leu Pro Glu Glu Asp Gln Ala Lys Leu Asp Ser Leu His Leu Pro Ala
        355                 360                 365

Gly Arg Ala Ala Tyr Ser Arg Glu Ser Leu Thr Ala Leu Ser Asp His
    370                 375                 380
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Met Leu Ala Thr Thr Asp Asp Leu His Glu Ala Arg Lys Arg Leu Phe
385                 390                 395                 400

Gly Val Asp Asp Ser Trp Ala Pro Pro Ala Glu Ala Ile Asn Ala Pro
                405                 410                 415

Val Gly Asn Pro Ser Val Asp Arg Thr Leu Lys Ile Val Gly Arg Tyr
            420                 425                 430

Leu Ser Ala Val Glu Ser Met Trp Gly Thr Pro Glu Val Ile His Val
        435                 440                 445

Glu His Val Arg Asp Gly Phe Thr Ser Glu Arg Met Ala Asp Glu Arg
    450                 455                 460

Asp Lys Ala Asn Arg Arg Arg Tyr Asn Asp Asn Gln Glu Ala Met Lys
465                 470                 475                 480

Lys Ile Gln Arg Asp Tyr Gly Lys Glu Gly Tyr Ile Ser Arg Gly Asp
                485                 490                 495
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Ile Val Arg Leu Asp Ala Leu Glu Leu Gln Gly Cys Ala Cys Leu Tyr
            500                 505                 510

Cys Gly Thr Thr Ile Gly Tyr His Thr Cys Gln Leu Asp His Ile Val
        515                 520                 525

Pro Gln Ala Gly Pro Gly Ser Asn Asn Arg Arg Gly Asn Leu Val Ala
    530                 535                 540

Val Cys Glu Arg Cys Asn Arg Ser Lys Ser Asn Thr Pro Phe Ala Val
545                 550                 555                 560

Trp Ala Gln Lys Cys Gly Ile Pro His Val Gly Val Lys Glu Ala Ile
                565                 570                 575

Gly Arg Val Arg Gly Trp Arg Lys Gln Thr Pro Asn Thr Ser Ser Glu
            580                 585                 590
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Asp Leu Thr Arg Leu Lys Lys Glu Val Ile Ala Arg Leu Arg Arg Thr
        595                 600                 605

Gln Glu Asp Pro Glu Ile Asp Glu Arg Ser Met Glu Ser Val Ala Trp
    610                 615                 620

Met Ala Asn Glu Leu His His Arg Ile Ala Ala Ala Tyr Pro Glu Thr
625                 630                 635                 640

Thr Val Met Val Tyr Arg Gly Ser Ile Thr Ala Ala Ala Arg Lys Ala
                645                 650                 655

Ala Gly Ile Asp Ser Arg Ile Asn Leu Ile Gly Glu Lys Gly Arg Lys
            660                 665                 670

Asp Arg Ile Asp Arg Arg His His Ala Val Asp Ala Ser Val Val Ala
        675                 680                 685

Leu Met Glu Ala Ser Val Ala Lys Thr Leu Ala Glu Arg Ser Ser Leu
    690                 695                 700
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Arg Gly Glu Gln Arg Leu Thr Gly Lys Glu Gln Thr Trp Lys Gln Tyr
705                 710                 715                 720

Thr Gly Ser Thr Val Gly Ala Arg Glu His Phe Glu Met Trp Arg Gly
                725                 730                 735

His Met Leu His Leu Thr Glu Leu Phe Asn Glu Arg Leu Ala Glu Asp
            740                 745                 750

Lys Val Tyr Val Thr Gln Asn Ile Arg Leu Arg Leu Ser Asp Gly Asn
        755                 760                 765

Ala His Thr Val Asn Pro Ser Lys Leu Val Ser His Arg Leu Gly Asp
    770                 775                 780

Gly Leu Thr Val Gln Gln Ile Asp Arg Ala Cys Thr Pro Ala Leu Trp
785                 790                 795                 800
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Cys Ala Leu Thr Arg Glu Lys Asp Phe Asp Glu Lys Asn Gly Leu Pro
805 810 815

Ala Arg Glu Asp Arg Ala Ile Arg Val His Gly His Glu Ile Lys Ser
820 825 830

Ser Asp Tyr Ile Gln Val Phe Ser Lys Arg Lys Lys Thr Asp Ser Asp
835 840 845

Arg Asp Glu Thr Pro Phe Gly Ala Ile Ala Val Arg Gly Gly Phe Val
850 855 860

Glu Ile Gly Pro Ser Ile His His Ala Arg Ile Tyr Arg Val Glu Gly
865 870 875 880

Lys Lys Pro Val Tyr Ala Met Leu Arg Val Phe Thr His Asp Leu Leu
885 890 895

Ser Gln Arg His Gly Asp Leu Phe Ser Ala Val Ile Pro Pro Gln Ser
900 905 910

Ile Ser Met Arg Cys Ala Glu Pro Lys Leu Arg Lys Ala Ile Thr Thr
915 920 925

Gly Asn Ala Thr Tyr Leu Gly Trp Val Val Val Gly Asp Glu Leu Glu
930 935 940

Ile Asn Val Asp Ser Phe Thr Lys Tyr Ala Ile Gly Arg Phe Leu Glu
945 950 955 960

Asp Phe Pro Asn Thr Thr Arg Trp Arg Ile Cys Gly Tyr Asp Thr Asn
965 970 975

Ser Lys Leu Thr Leu Lys Pro Ile Val Leu Ala Ala Glu Gly Leu Glu
980 985 990

Asn Pro Ser Ser Ala Val Asn Glu Ile Val Glu Leu Lys Gly Trp Arg
995 1000 1005

Val Ala Ile Asn Val Leu Thr Lys Val His Pro Thr Val Val Arg

1010 1015 1020

Arg Asp Ala Leu Gly Arg Pro Arg Tyr Ser Ser Arg Ser Asn Leu

1025 1030 1035

Pro Thr Ser Trp Thr Ile Glu

1040 1045

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<212> PRT

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<400> 9

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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe

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

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp

130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Arg
180 185 190

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
195 200 205

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
210 215 220

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
225 230 235 240

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
245 250 255

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
260 265 270

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

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
290 295 300

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
305 310 315 320

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
325 330 335

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr

340 345 350

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
355 360 365

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
370 375 380

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
385 390 395 400

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
405 410 415

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
420 425 430

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
435 440 445

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
450 455 460

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
465 470 475 480

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
485 490 495

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
500 505 510

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
515 520 525

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
530 535 540

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln

545 550 555 560

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
565 570 575

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
580 585 590

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
595 600 605

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
610 615 620

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
625 630 635 640

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
645 650 655

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
660 665 670

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
675 680 685

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
690 695 700

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
705 710 715 720

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
725 730 735

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
740 745 750

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg

755 760 765

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
770 775 780

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
785 790 795 800

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala Lys
805 810 815

Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser
820 825 830

Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly Glu
835 840 845

Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu Thr Gly Glu Ile
850 855 860

```
Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser
865                 870                 875                 880

Met Pro Gln Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly Gly
                885                 890                 895

Phe Ser Lys Glu Ser Ile Leu Pro Lys Arg Asn Ser Asp Lys Leu Ile
            900                 905                 910

Ala Arg Lys Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser
        915                 920                 925

Pro Thr Val Ala Tyr Ser Val Leu Val Val Ala Lys Val Glu Lys Gly
    930                 935                 940

Lys Ser Lys Lys Leu Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile
945                 950                 955                 960

Met Glu Arg Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala
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965 970 975

Lys Gly Tyr Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys
980 985 990

Tyr Ser Leu Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser
995 1000 1005

Ala Gly Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys
1010 1015 1020

Tyr Val Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys
1025 1030 1035

Gly Ser Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln
1040 1045 1050

His Lys His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe
1055 1060 1065

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Ser Lys  Arg Val Ile Leu Ala  Asp Ala Asn Leu Asp  Lys Val Leu
    1070                 1075                 1080

Ser Ala  Tyr Asn Lys His Arg  Asp Lys Pro Ile Arg  Glu Gln Ala
    1085                 1090                 1095

Glu Asn  Ile Ile His Leu Phe  Thr Leu Thr Asn Leu  Gly Ala Pro
    1100                 1105                 1110

Ala Ala  Phe Lys Tyr Phe Asp  Thr Thr Ile Asp Arg  Lys Arg Tyr
    1115                 1120                 1125

Thr Ser  Thr Lys Glu Val Leu  Asp Ala Thr Leu Ile  His Gln Ser
    1130                 1135                 1140

Ile Thr  Gly Leu Tyr Glu Thr  Arg Ile Asp Leu Ser  Gln Leu Gly
    1145                 1150                 1155

Gly Asp
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1160

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ccccccna 8

<210> 11

<211> 8

<212> DNA

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<400> 11

cccccaa 8

<210> 12

<211> 5

<212> DNA

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<223> PAM

<400> 12

ccccc 5

<210> 13

<211> 8

<212> DNA

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<220>

<223> PAM

<220>

<221> misc_feature

<222> (6)..(7)

<223> n is a, c, g, or t

<400> 13

ccccnna 8

<210> 14

<211> 6

<212> DNA

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<400> 14

cccccc 6

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<211> 6

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<222> (1)..(1)

<223> n is a, c, g, or t

<400> 15

ncccccc 6

<210> 16

<211> 8

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<221> misc_feature

<222> (1)..(1)

<223> n is a, c, g, or t

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<221> misc_feature

<222> (7)..(7)

<223> n is a, c, g, or t

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<210> 17

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<220>

<221> misc_feature

<222> (2)..(2)

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cnccccac 8

<210> 18

<211> 8

<212> DNA

<213> Artificial Sequence

<220>

<223> PAM

<400> 18

ccccccag 8

<210> 19

<211> 8

<212> DNA

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<220>

<223> PAM

<400> 19

cccccat 8

<210> 20

<211> 8

<212> DNA

<213> Artificial Sequence

<220>

<223> PAM

<400> 20

ccccccac 8

<210> 21

<211> 8

<212> DNA

<213> Artificial Sequence

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<223> PAM

<400> 21

atccccaa 8

<210> 22

<211> 8

<212> DNA

<213> Artificial Sequence

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<223> PAM

<400> 22

acggccaa 8

<210> 23

<211> 11

<212> PRT

<213> Artificial Sequence

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<223> RuvC-1 domain motif

<400> 23

Ile Gly Leu Asp Ile Gly Ile Thr Ser Ile Gly

1 5 10

<210> 24

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> RuvC-I domain motif

<400> 24

Ile Gly Leu Asp Ile Gly Ile Thr Ser Ile Gly Trp Ala Val Ile Asn

1 5 10 15

Leu Asp

<210> 25

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Bridge domain motif

<400> 25

Arg Ser Ala Arg Arg
1 5

<210> 26

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Bridge domain motif

<400> 26

Pro Arg Arg Leu Ala Arg Ser Ala Arg Arg Arg Leu Arg Arg Arg Lys
1 5 10 15

His Arg Leu Glu Arg Ile Arg Arg Leu
20 25

<210> 27

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> alpha-helical/recognition lobe domain motif

<400> 27

Trp Gln Leu Arg

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<210> 28

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> alpha-helical/recognition lobe domain

<400> 28

His Leu Ala Lys Arg Arg Gly

1 5

<210> 29

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> alpha-helical/recognition lobe domain

<400> 29

Leu Ala Arg Ile Leu Leu His Leu Ala Lys Arg Arg Gly
1 5 10

<210> 30

<211> 5

<212> PRT

<213> Artificial Sequence

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<223> alpha-helical/recognition lobe domain

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Ile Phe Ala Lys Gln
1 5

<210> 31

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> alpha-helical/recognition lobe domain

<400> 31

Glu Ile Lys Leu Ile Phe Ala Lys Gln
1 5

<210> 32

<211> 6

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<220>

<223> alpha-helical/recognition lobe domain

<400> 32

Ile Trp Ala Ser Gln Arg

1 5

<210> 33

<211> 16

<212> PRT

<213> Artificial Sequence

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<223> alpha-helical/recognition lobe domain motif

<400> 33

Lys Val Gly Phe Cys Thr Phe Glu Pro Lys Glu Lys Arg Ala Pro Lys

1 5 10 15

<210> 34

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> alpha-helical/recognition lobe domain motif

<400> 34

Phe Thr Val Trp Glu His Ile Asn Lys Leu Arg Leu
1 5 10

<210> 35

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> RuvC-II domain motif

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Ile Ala Asn Pro Val Val Met Arg Ala Leu Thr Gln
1 5 10

<210> 36

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> RuvC-II domain motif

<400> 36

Ile Ala Asn Pro Val Val Met Arg Ala Leu Thr Gln Ala Arg Lys Val
1 5 10 15

Val Asn Ala Ile Ile Lys Lys Tyr Gly
20 25

<210> 37

<211> 4

<212> PRT

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<223> RuvC-II domain motif

<400> 37

Glu Leu Ala Arg
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<210> 38

<211> 8

<212> PRT

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<223> RuvC-II domain motif

<400> 38

Ile His Ile Glu Leu Ala Arg Glu
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<210> 39

<211> 7

<212> PRT

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<220>

<223> HNH domain motif

<400> 39

Gln Asn Gly Lys Cys Ala Tyr
1 5

<210> 40

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> HNH domain motif

<400> 40

Ile Val Lys Phe Lys Leu Trp Ser Glu Gln Asn Gly Lys Cys Ala Tyr
1 5 10 15

<210> 41

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> HNH domain motif

<400> 41

Val Asp His Val Ile Pro

1 5

<210> 42

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> HNH domain motif

<400> 42

Val Asp His Val Ile Pro Tyr Ser Arg Ser Leu Asp Asp Ser Tyr Thr

1 5 10 15

Asn Lys Val Leu

20

<210> 43

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> RuvC-III domain motif

<400> 43

Asp Thr Arg Tyr Ile Ser Arg Phe Leu Ala Asn
1 5 10

<210> 44

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> RuvC-III domain motif

<400> 44

Val Tyr Thr Val Asn Gly Arg Ile Thr Ala His Leu Arg Ser Arg Trp
1 5 10 15

<210> 45

<211> 6

<212> PRT

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<220>

<223> RuvC-III domain motif

<400> 45

His His Ala Val Asp Ala

1 5

<210> 46

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> RuvC-III domain motif

<400> 46

His His Ala Val Asp Ala Ala Ile Val Ala

1 5 10

<210> 47

<211> 8

<212> DNA

<213> Artificial Sequence

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<223> PAM

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<221> misc_feature

<222> (1)..(4)

<223> n is a, c, g, or t

<220>

<221> misc_feature

<222> (6)..(7)

<223> n is a, c, g, or t

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nnnncnna 8

<210> 48

<211> 8

<212> DNA

<213> Artificial Sequence

<220>

<223> PAM

<220>

<221> misc_feature

<222> (1)..(4)

<223> n is a, c, g, or t

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<210> 49

<211> 8

<212> DNA

<213> Artificial Sequence

<220>

<223> PAM

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<222> (1)..(4)

<223> n is a, c, g, or t

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<210> 50

<211> 8

<212> DNA

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<223> PAM

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<222> (1)..(4)

<223> n is a, c, g, or t

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<210> 51

<211> 8

<212> DNA

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<223> PAM

<220>

<221> misc_feature

<222> (1)..(4)

<223> n is a, c, g, or t

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nnnnccaa 8

<210> 52

<211> 8

<212> DNA

<213> Artificial Sequence

<220>

<223> PAM

<220>

<221> misc_feature

<222> (1)..(4)

<223> n is a, c, g, or t

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<222> (6)..(6)

<223> n is a, c, g, or t

<400> 52

nnnncnaa 8

<210> 53

<211> 8

<212> DNA

<213> Artificial Sequence

<220>

<223> PAM

<220>

<221> misc_feature

<222> (1)..(4)

<223> n is a, c, g, or t

<400> 53

nnnncmca 8

<210> 54

<211> 30

<212> RNA

<213> Artificial Sequence

<220>

<223> tracrRNA 5' hairpin

<400> 54

aagggcuuuc ugccuauagg cagacugccc 30

<210> 55

<211> 25

<212> RNA

<213> Artificial Sequence

<220>

<223> tracrRNA 'middle' hairpin

<400> 55

guggcguugg ggaucgccua ucgcc 25

<210> 56

<211> 36

<212> RNA

<213> Artificial Sequence

<220>

<223> tracrRNA 3' hairpin

<400> 56

cgcuuucuuc gggcauuccc cacucuuagg cguuuu 36

<210> 57

<211> 55

<212> RNA

<213> Artificial Sequence

<220>

<223> tracrRNA 5' hairpin and middle hairpin

<400> 57

aagggcuuuc ugccuauagg cagacugccc guggcguugg ggaucgccua ucgcc 55

<210> 58

<211> 91

<212> RNA

<213> Artificial Sequence

<220>

<223> tracrRNA 5' hairpin, middle hairpin and 3' hairpin.

<400> 58

aagggcuuuc ugccuauagg cagacugccc guggcguugg ggaucgccua ucgcccgcuu 60

ucuucgggca uuccccacuc uuaggcguuu u 91

<210> 59

<211> 81

<212> DNA

<213> Artificial Sequence

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<223> BG6494

<220>

<221> misc_feature

<222> (35)..(41)

<223> n is a, c, g, or t

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aaatgaagtt ttaaatcaat c 81

<210> 60

<211> 81

<212> DNA

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<223> BG6495

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ttttaattta aaaggatcta g 81

<210> 61

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> BG7356

<400> 61

tcgtcggcag cgtcagatgt gtataagaga cagt 34

<210> 62

<211> 33

<212> DNA

<213> Artificial Sequence

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<223> BG7357

<400> 62

ctgtctctta tacacatctg acgctgccga cga 33

<210> 63

<211> 16

<212> DNA

<213> Artificial Sequence

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<223> BG7358

<400> 63

tcgtcggcag cgtcag 16

<210> 64

<211> 53

<212> DNA

<213> Artificial Sequence

<220>

<223> BG7359

<400> 64

gtctcgtggg ctcggagatg tgtataagag acaggaccat gattacgcca agc 53

<210> 65

<211> 61

<212> DNA

<213> Artificial Sequence

<220>

<223> BG7616

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c 61

<210> 66

<211> 82

<212> DNA

<213> Artificial Sequence

<220>

<223> BG8157

<400> 66

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aaaatgaagt tttaaatcaa tc 82

<210> 67

<211> 82

<212> DNA

<213> Artificial Sequence

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<223> BG8158

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aaaatgaagt tttaaatcaa tc 82

<210> 68

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<212> DNA

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<223> BG8159

<400> 68

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aaaatgaagt tttaaatcaa tc 82

<210> 69

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<223> BG8160

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aaaatgaagt tttaaatcaa tc 82

<210> 70

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<223> BG8161

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<222> (35)..(38)

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<222> (40)..(41)

<223> n is a, c, g, or t

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aaatgaagtt ttaaatcaat c 81

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<212> DNA

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<223> BG8363

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<210> 72

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<223> BG8364

<400> 72

cgggattgac ttttaaaaaa gg 22

<210> 73

<211> 82

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<223> BG8763

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<210> 74

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<213> Artificial Sequence

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<223> BG8764

<400> 74

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aaaatgaagt tttaaatcaa tc 82

<210> 75

<211> 82

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<223> BG8765

<400> 75

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aaaatgaagt tttaaatcaa tc 82

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<223> BG8766

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<210> 77

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<223> BG8767

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aaaatgaagt tttaaatcaa tc 82

<210> 78

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<223> BG8768

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aaaatgaagt tttaaatcaa tc 82

<210> 79

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<223> BG8769

<400> 79

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<210> 80

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<223> BG8770

<400> 80

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aaaatgaagt tttaaatcaa tc 82

<210> 81

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<223> BG8771

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aaaatgaagt tttaaatcaa tc 82

<210> 82

<211> 82

<212> DNA

<213> Artificial Sequence

<220>

<223> BG8772

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aaaatgaagt tttaaatcaa tc 82

<210> 83

<211> 82

<212> DNA

<213> Artificial Sequence

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<223> BG8773

<400> 83

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aaaatgaagt tttaaatcaa tc 82

<210> 84

<211> 82

<212> DNA

<213> Artificial Sequence

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<223> BG8774

<400> 84

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aaaatgaagt tttaaatcaa tc 82

<210> 85

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<223> BG8775

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aaaatgaagt tttaaatcaa tc 82

<210> 86

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<212> DNA

<213> Artificial Sequence

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<223> BG8776

<400> 86

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aaaatgaagt tttaaatcaa tc 82

<210> 87

<211> 82

<212> DNA

<213> Artificial Sequence

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<223> BG8777

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<210> 88

<211> 82

<212> DNA

<213> Artificial Sequence

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<223> BG8778

<400> 88

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aaaatgaagt tttaaatcaa tc 82

<210> 89

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<213> Artificial Sequence

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<223> BG6574

<400> 89

aagcttgaaa taatacgact cactatagg 29

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<211> 22

<212> DNA

<213> Artificial Sequence

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<223> BG6576

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aaaaaagacc ttgacgtttt cc 22

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<223> BG9307

<400> 91

aagcttgaaa taatacgact cactataggt gagattatca aaaaggatct tcacgtc 57

<210> 92

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<212> DNA

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<223> BG9309

<400> 92

aaaacgccta agagtgggga atg 23

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<223> BG9310

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aaaaggcgat aggcgatcc 19

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<211> 23

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<223> BG9311

<400> 94

aaaacgggtc agtctgccta tag 23

<210> 95

<211> 57

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<223> BG9308

<400> 95

aagcttgaaa taatacgact cactataggt gagattatca aaaaggatct tcacgtc 57

<210> 96

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<223> BG10118

<400> 96

aagcttgaaa taatacgact cactatagga gattatcaaa aaggatcttc acgtca 56

<210> 97

<211> 60

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<223> BG10119

<400> 97

aagcttgaaa taatacgact cactatagga agattatcaa aaaggatctt cacgtcatag 60

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<211> 58

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