SEQUENCE LISTING

<110> THE BROAD INSTITUTE, INC.
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

<120> CRISPR ENZYME MUTATIONS REDUCING OFF-TARGET EFFECTS

<130> 47627.99.2017

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<150> 62/269,876
<151> 2015-12-18

<150> 62/255,256
<151> 2015-11-13

<150> 62/237,360
<151> 2015-10-05

<150> 62/207,312
<151> 2015-08-19

<150> 62/181,453
<151> 2015-06-18

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<170> PatentIn version 3.5

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Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
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35 40 45

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nnnnnnnnnn nnnggng 17

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<223> a, c, t, g, unknown or other

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gctacaaaga taaggcttca tgccgaaatc aacaccctgt cattttatgg cagggtgttt 120

tcgttattta atttttt 137

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gcttcatgcc gaaatcaaca ccctgtcatt ttatggcagg gtgttttcgt tatttaattt 120

ttt 123

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gcttcatgcc gaaatcaaca ccctgtcatt ttatggcagg gtgtttttt 110

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cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tt 102

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cgttatcaac ttgaaaaagt gttttttt 88

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cgttatcatt tttttt 76

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<211> 7
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<213> Simian virus 40

<400> 24
Pro Lys Lys Lys Arg Lys Val
1 5

<210> 25
<211> 16
<212> PRT
<213> Unknown

<220>
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<223> /note="Description of Unknown:
Nucleoplasmin bipartite NLS sequence"

<400> 25
Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys
1 5 10 15

<210> 26
<211> 9
<212> PRT
<213> Unknown

<220>
<221> source
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Pro Ala Ala Lys Arg Val Lys Leu Asp
1 5

<210> 27
<211> 11
<212> PRT
<213> Unknown

<220>
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<223> /note="Description of Unknown:
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<400> 27
Arg Gln Arg Arg Asn Glu Leu Lys Arg Ser Pro
1 5 10

<210> 28
<211> 38
<212> PRT
<213> Homo sapiens

<400> 28
Asn Gln Ser Ser Asn Phe Gly Pro Met Lys Gly Gly Asn Phe Gly Gly

1 5 10 15

Arg Ser Ser Gly Pro Tyr Gly Gly Gly Gly Gln Tyr Phe Ala Lys Pro
20 25 30

Arg Asn Gln Gly Gly Tyr
35

<210> 29
<211> 42
<212> PRT
<213> Unknown

<220>
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IBB domain from importin-alpha sequence"

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Arg Met Arg Ile Glx Phe Lys Asn Lys Gly Lys Asp Thr Ala Glu Leu
1 5 10 15

Arg Arg Arg Arg Val Glu Val Ser Val Glu Leu Arg Lys Ala Lys Lys
20 25 30

Asp Glu Gln Ile Leu Lys Arg Arg Asn Val
35 40

<210> 30
<211> 8
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Myoma T protein sequence"

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Val Ser Arg Lys Arg Pro Arg Pro
1 5

<210> 31
<211> 8
<212> PRT
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<220>
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Myoma T protein sequence"

<400> 31
Pro Pro Lys Lys Ala Arg Glu Asp
1 5

<210> 32
<211> 8
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<400> 32
Pro Gln Pro Lys Lys Lys Pro Leu
1 5

<210> 33
<211> 12
<212> PRT
<213> Mus musculus

<400> 33
Ser Ala Leu Ile Lys Lys Lys Lys Lys Met Ala Pro
1 5 10

<210> 34
<211> 5
<212> PRT
<213> Influenza virus

<400> 34
Asp Arg Leu Arg Arg
1 5

<210> 35
<211> 7
<212> PRT
<213> Influenza virus

<400> 35
Pro Lys Gln Lys Lys Arg Lys
1 5

<210> 36
<211> 10
<212> PRT
<213> Hepatitis delta virus

<400> 36
Arg Lys Leu Lys Lys Lys Ile Lys Lys Leu
1   5   10

<210> 37
<211> 10
<212> PRT
<213> Mus musculus

<400> 37
Arg Glu Lys Lys Lys Phe Leu Lys Arg Arg
1   5   10

<210> 38
<211> 20
<212> PRT
<213> Homo sapiens

<400> 38
Lys Arg Lys Gly Asp Glu Val Asp Gly Val Asp Glu Val Ala Lys Lys
1   5   10   15

Lys Ser Lys Lys
            20

<210> 39
<211> 17
<212> PRT
<213> Homo sapiens

<400> 39
Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys
1   5   10   15

Lys

<210> 40
<211> 12
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Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
1   5   10

<210> 41
<211> 7
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Ala Glu Ala Ala Ala Lys Ala
1   5

<210> 42
<211> 5
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Gly Gly Gly Gly Ser
1   5

<210> 43
<211> 10
<212> PRT
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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1   5   10

<210> 44
<211> 20
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<400> 44
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
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Gly Gly Gly Ser
20

<210> 45
<211> 25
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<220>
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<400> 45
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25

<210> 46
<211> 35
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polypeptide"

<400> 46
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1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
20 25 30

Gly Gly Ser

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<210> 47
<211> 40
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<213> Artificial Sequence

<220>
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1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
20 25 30

Gly Gly Ser Gly Gly Gly Gly Ser
35 40

<210> 48
<211> 50
<212> PRT
<213> Artificial Sequence

<220>
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<400> 48
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1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
20 25 30

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
35 40 45

Gly Ser
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<210> 49

<211> 55
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polypeptide"

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1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
20 25 30

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
35 40 45

Gly Ser Gly Gly Gly Gly Ser
50 55

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<223> Ahx

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<210> 52
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<400> 52
gagtcctagc aggagaagaa   20

<210> 53
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<400> 53
gagtctaagc agaagaagaa   20

<210> 54
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<400> 54
guuuuagagc ua   12

<210> 55
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<212> PRT
<213> Unknown

<220>
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LAGLIDADG family motif peptide"

<400> 55
Leu Ala Gly Leu Ile Asp Ala Asp Gly
1 5

<210> 56
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gagtccgagc agaagaagaa ggg 23

<210> 57
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<210> 58
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gagttagagc agaagaagaa agg 23

<210> 59
<211> 23

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gggtgggggg agtttgctcc tgg 23

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<220>
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<211> 23
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<220>
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cgggggaggg agtttgctcc tgg 23

<210> 62
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<210> 63
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ggtgagtgag tgtgtgtgtg agg 23

<210> 64
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
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agtgagtgag tgtgtgtgtg ggg 23

<210> 65
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<220>
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gctgagtgag tgtatgcgtg tgg 23

<210> 66
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gttgagtgaa tgtgtgcgtg agg 23

<210> 67
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tgtgggtgag tgtgtgcgtg agg 23

<210> 68
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<210> 69
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<220>
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gacctcccca tagcctggcc agggagg 27

<210> 70
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<220>
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<220>
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oligonucleotide"

<400> 71
ggcctcccaa agccaggcca ggggga 26

<210> 72
<211> 32
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<220>
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primer"

<400> 72
atggtctcac cggtgccacc atggactata ag 32

<210> 73
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<220>
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primer"

<400> 73
atggtctcag gcgaacagcc gcgagagaat gaagcggat 39

<210> 74
<211> 38
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<220>
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primer"

<400> 74
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<210> 75
<211> 40
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<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic primer"

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<400> 495
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<400> 519
cgtgagtgag tgtgtacctg ggg 23

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<213> Staphylococcus aureus

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1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

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

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    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 Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270
```

```
Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

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

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
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                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700
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Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720


His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735


Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750


Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765


Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780


Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800


Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815


Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830


Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845


Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860


Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880


Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895


Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910


Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
```

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        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Thr
                965                 970                 975

Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly Phe Ile
            980                 985                 990

Lys Arg Gln Leu Val Glu Thr Arg  Gln Ile Thr Lys His  Val Ala Gln
        995                 1000                1005

Ile Leu  Asp Ser Arg Met Asn  Thr Lys Tyr Asp Glu  Asn Asp Lys
    1010                1015                1020

Leu Ile  Arg Glu Val Lys Val  Ile Thr Leu Lys Ser  Lys Leu Val
    1025                1030                1035

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

Asn Asn  Tyr His His Ala His  Asp Ala Tyr Leu Asn  Ala Val Val
    1055                1060                1065

Gly Thr  Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
    1070                1075                1080

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1085                1090                1095

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1100                1105                1110

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1115                1120                1125
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Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1130                1135                1140

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1145                1150                1155

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1160                1165                1170

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1175                1180                1185

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1190                1195                1200

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1205                1210                1215

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1220                1225                1230

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1235                1240                1245

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1250                1255                1260

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1265                1270                1275

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1280                1285                1290

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1295                1300                1305

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1310                1315                1320

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
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```
    1325                1330                1335

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1340                1345                1350

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1355                1360                1365

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1370                1375                1380

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1385                1390                1395

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1400                1405                1410

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
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Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
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<400> 521
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