

SEQUENCE LISTING

<110> Regeneron Pharmaceuticals, Inc.

<120> CRISPR/CAS SCREENING PLATFORM TO IDENTIFY GENETIC MODIFIERS OF
TAU SEEDING OR AGGREGATION

<130> 057766-544635

<150> US 62/820,086

<151> 2019-03-18

<160> 43

<170> PatentIn version 3.5

<210> 1

<211> 31

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 1

Gln	Thr	Ala	Pro	Val	Pro	Met	Pro	Asp	Leu	Lys	Asn	Val	Lys	Ser	Lys
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Ile	Gly	Ser	Thr	Glu	Asn	Leu	Lys	His	Gln	Pro	Gly	Gly	Gly	Lys
			20					25					30	

<210> 2

<211> 31

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Val	Gln	Ile	Ile	Asn	Lys	Lys	Leu	Asp	Leu	Ser	Asn	Val	Gln	Ser	Lys
1				5					10					15	

Cys	Gly	Ser	Lys	Asp	Asn	Ile	Lys	His	Val	Pro	Gly	Gly	Gly	Ser
			20					25					30	

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<400> 3

Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser Lys
1 5 10 15

Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln
20 25 30

<210> 4
<211> 32
<212> PRT
<213> Artificial Sequence

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<223> Synthetic

<400> 4

Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser
1 5 10 15

Lys Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn
20 25 30

<210> 5
<211> 93
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<400> 5

cagacagccc ccgtgcccac gccagacctg aagaatgtca agtccaagat cggctccact 60

gagaacctga agcaccagcc gggaggcgagg aag 93

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<211> 93
<212> DNA
<213> Artificial Sequence

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<223> Synthetic

<400> 6

gtgcagataa ttaataagaa gctggatctt agcaacgtcc agtccaagtg tggctcaaag 60

gataatatca aacacgtccc gggaggcggc agt 93

<210> 7

<211> 93

<212> DNA

<213> Artificial Sequence

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gtgcaaatag tctacaaacc agttgacctg agcaaggtga cctccaagtg tggctcatta 60

ggcaacatcc atcataaacc aggaggtggc cag 93

<210> 8

<211> 96

<212> DNA

<213> Artificial Sequence

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<223> Synthetic

<400> 8

gtggaagtaa aatctgagaa gcttgacttc aaggacagag tccagtcgaa gattgggtcc 60

ctggacaata tcaccacgt ccctggcgga ggaaat 96

<210> 9

<211> 133

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 9

Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys Ser
1 5 10 15

Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly Gly Lys
20 25 30

Val Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser Asn Val Gln Ser Lys
35 40 45

Cys Gly Ser Lys Asp Asn Ile Lys His Val Pro Gly Gly Gly Ser Val
50 55 60

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

Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln Val Glu
85 90 95

Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser Lys Ile
100 105 110

Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn Lys Lys
115 120 125

Ile Glu Thr His Lys
130

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gatcttagca acgtccagtc caagtgtggc tcaaaggata atatcaaaca cgtcccggga 180
ggcggcagtg tgcaaatagt ctacaaacca gttgacctga gcaaggtgac ctccaagtgt 240
ggctcattag gcaacatcca tcataaacca ggaggtggcc aggtggaagt aaaatctgag 300
aagcttgact tcaaggacag agtccagtcg aagattgggt ccctggacaa tatcaccac 360
gtccctggcg gaggaataa aaagattgaa acccacaag 399

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<211> 133

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 11

Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys Ser
1 5 10 15

Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly Gly Lys
20 25 30

Val Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser Asn Val Gln Ser Lys
35 40 45

Cys Gly Ser Lys Asp Asn Ile Lys His Val Ser Gly Gly Gly Ser Val
50 55 60

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

Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln Val Glu
85 90 95

Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser Lys Ile
100 105 110

Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn Lys Lys
115 120 125

Ile Glu Thr His Lys
130

<210> 12
<211> 399
<212> DNA
<213> Artificial Sequence

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<223> Synthetic

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gatcttagca acgtccagtc caagtgtggc tcaaaggata atatcaaaca cgtctcggga	180
ggcggcagtg tgcaaatagt ctacaaacca gttgacctga gcaaggtgac ctccaagtgt	240
ggctcattag gcaacatcca tcataaacca ggaggtggcc aggtggaagt aaaatctgag	300
aagcttgact tcaaggacag agtccagtcg aagattgggt ccctggacaa tatcaccac	360
gtccctggcg gaggaaataa aaagattgaa acccacaag	399

$\langle 210 \rangle$	13
$\langle 211 \rangle$	238
$\langle 212 \rangle$	PRT
$\langle 213 \rangle$	Artificial Sequence

<400> 13

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
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Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
50 55 60

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140

Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225 230 235

<210> 14
<211> 714
<212> DNA
<213> Artificial Sequence

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<223> Synthetic

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gacgtaaacg gccacaagtt cagcgtgtcc ggcgagggcg agggcgatgc cacctacggc 120
aagctgaccc tgaagttcat ctgcaccacc ggcaagctgc ccgtgccctg gcccaccctc 180
gtgaccaccc tgacctgggg cgtgcagtgc ttcagccgct accccgacca catgaagcag 240
cacgacttct tcaagtccgc catgcccga ggctacgtcc aggagcgcac catcttcttc 300
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aaccgcatcg agctgaaggg catcgacttc aaggaggacg gcaacatcct ggggcacaag 420
ctggagtaca actacatcag ccacaacgtc tatatcaccg ccgacaagca gaagaacggc 480

atcaaggcca acttcaagat ccgccacaac atcgaggacg gcagcgtgca gctcgccgac 540
 cactaccagc agaacacccc catcggcgac ggccccgtgc tgctgcccga caaccactac 600
 ctgagcacc agtccgccct gagcaaagac cccaacgaga agcgcgatca catggtcctg 660
 ctggagtctg tgaccgccgc cgggatcact ctcggcatgg acgagctgta caag 714

<210> 15
 <211> 236
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 15

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
 20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
 35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60

Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln
 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
 85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
 100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
 115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu
225 230 235

<210> 16
<211> 708
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

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aagctgaccc tgaagttcat ctgcaccacc ggcaagctgc ccgtgccctg gcccaccctc 180
gtgaccacct tcggctacgg cctgcagtgc ttgcccgcct accccgacca catgaagcag 240
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aaggacgacg gcaactacaa gacccgcgcc gaggtgaagt tcgagggcga caccctggtg 360
aaccgcatcg agctgaaggg catcgacttc aaggaggacg gcaacatcct ggggcacaag 420
ctggagtaca actacaacag ccacaacgtc tatatcatgg ccgacaagca gaagaacggc 480
atcaaggtga acttcaagat ccgccacaac atcgaggacg gcagcgtgca gctcgccgac 540
cactaccagc agaacacccc catcggcgac ggccccgtgc tgctgcccga caaccactac 600

ctgagctacc agtccgccct gagcaaagac cccaacgaga agcgcgatca catggtcctg 660

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<210> 17

<211> 77

<212> RNA

<213> Artificial Sequence

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<223> Synthetic

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ggcaccgagu cggugcu 77

<210> 18

<211> 82

<212> RNA

<213> Artificial Sequence

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<400> 18

guuggaacca uucaaaacag cauagcaagu uaaaauaagg cuaguccguu aucaacuuga 60

aaaaguggca ccgagucggu gc 82

<210> 19

<211> 76

<212> RNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 19

guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc cguuaucaac uugaaaaagu 60

ggcaccgagu cggugc 76

<210> 20

<211> 86

<212> RNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 20

guuuuagagc uaugcuggaa acagcauagc aaguuuuuuu aaggcuaguc cguuaucaac 60

uugaaaaagu ggcaccgagu cggugc 86

<210> 21

<211> 1392

<212> PRT

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<220>

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<222> (1)..(1384)

<223> Cas9

<220>

<221> MISC_FEATURE

<222> (1385)..(1392)

<223> FLAG

<400> 21

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
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
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
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

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
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 Arg

965

970

975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
 980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe
 995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala
 1010 1015 1020

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe
 1025 1030 1035

Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala
 1040 1045 1050

Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu
 1055 1060 1065

Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val
 1070 1075 1080

Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr
 1085 1090 1095

Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys
 1100 1105 1110

Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
 1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
 1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
 1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
 1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys

1370

1375

1380

Lys Asp Tyr Lys Asp Asp Asp Asp Lys
 1385 1390

<210> 22
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 <212> DNA
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 <222> (1)..(4152)
 <223> Cas9

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 <222> (4153)..(4176)
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 gccacccggc tgaagagaac cgccagaaga agatacacca gacggaagaa ccggatctgc 240
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aacctgccc aacgagaaggt gctgcccag cacagcctgc tgtacgagta cttcaccgtg	1560
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agcggcgagc agaaaaaggc catcgtggac ctgctgttca agaccaaccg gaaagtgacc	1680
gtgaagcagc tgaaagagga ctacttcaag aaaatcgagt gcttcgactc cgtggaaatc	1740
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<220>
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guuuuagagc uaugcu	16

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<400> 24	
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gugcuuu	67

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 <222> (2)..(21)
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gnnnnnnnnnn nnnnnnnnnn ngg	23

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 <223> n = A, T, C, or G

<400> 26
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<220>
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 <222> (3)..(23)
 <223> n = A, T, C, or G

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<220>
 <223> Synthetic

<400> 28
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 gucggugcuu uu 72

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<220>
 <223> Synthetic

<400> 29
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 aaaaguggca ccgagucggu gc 82

<210> 30
 <211> 83
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 30
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 ggcaccgagu cggugcuuuu uu 83

<210> 31
 <211> 80
 <212> RNA
 <213> Artificial Sequence

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 <223> Synthetic

<400> 31
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 ggcaccgagu cggugcuuuu 80

<210> 32
 <211> 92
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Synthetic

<400> 32
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 uugaaaaagu ggcaccgagu cggugcuuuu uu 92

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<223> Synthetic

<400> 33

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34

<210> 34

<211> 137

<212> RNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 34

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60

aaggcuaguc cguuaucaac uaggccaaca ugaggaucaac ccaugucugc agggccaagu

120

ggcaccgagu cggugcu

137

<210> 35

<211> 157

<212> RNA

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<220>

<221> misc_feature

<222> (1)..(20)

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

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nnnnnnnnnnn nnnnnnnnnnn guuuuagagc uaggccaaca ugaggaucaac ccaugucugc

60

agggccuagc aaguuaaaau aaggcuaguc cguuaucaac uaggccaaca ugaggaucaac

120

ccaugucugc agggccaagu ggcaccgagu cggugcu

157

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<223> Synthetic

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<223> VP64

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Lys Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
35 40 45

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
50 55 60

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
65 70 75 80

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
85 90 95

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
100 105 110

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
115 120 125

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
130 135 140

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
145 150 155 160

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
165 170 175

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
180 185 190

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
195 200 205

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
210 215 220

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
225 230 235 240

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
245 250 255

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

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
275 280 285

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
290 295 300

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
305 310 315 320

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
325 330 335

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
340 345 350

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
355 360 365

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
370 375 380

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
385 390 395 400

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
405 410 415

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
420 425 430

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
435 440 445

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
450 455 460

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
465 470 475 480

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
485 490 495

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
500 505 510

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
515 520 525

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
530 535 540

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
545 550 555 560

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
565 570 575

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
580 585 590

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
595 600 605

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
610 615 620

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
625 630 635 640

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
645 650 655

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
660 665 670

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
675 680 685

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
690 695 700

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
705 710 715 720

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
725 730 735

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
740 745 750

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
755 760 765

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
770 775 780

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
785 790 795 800

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
805 810 815

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
820 825 830

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
835 840 845

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

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Ala Arg
865 870 875 880

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
885 890 895

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
900 905 910

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
915 920 925

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
930 935 940

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
945 950 955 960

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
965 970 975

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
980 985 990

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
995 1000 1005

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu
1010 1015 1020

Phe Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile
1025 1030 1035

Ala Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe
1040 1045 1050

Phe Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu
1055 1060 1065

Ala Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly
1070 1075 1080

Glu Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr
1085 1090 1095

Val Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys Lys
1100 1105 1110

Thr Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro
1115 1120 1125

Lys Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp
1130 1135 1140

Pro Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser
1145 1150 1155

Val Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu
1160 1165 1170

Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser
1175 1180 1185

Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr
1190 1195 1200

Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser
1205 1210 1215

Leu Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala
1220 1225 1230

Gly Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr
1235 1240 1245

Val Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly
1250 1255 1260

Ser Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His
1265 1270 1275

Lys His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser
1280 1285 1290

Lys Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser
1295 1300 1305

Ala Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu
1310 1315 1320

Asn Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala
1325 1330 1335

Ala Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr
1340 1345 1350

Ser Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile
1355 1360 1365

Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly
1370 1375 1380

Asp Ser Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
1385 1390 1395

Gly Gly Ser Gly Pro Lys Lys Lys Arg Lys Val Ala Ala Ala Gly
1400 1405 1410

Ser Gly Arg Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu
1415 1420 1425

Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser
1430 1435 1440

Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
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Leu Asp Asp Phe Asp Leu Asp Met Leu Ile Asn Cys Thr
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<222> (151)..(155)
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<223> P65

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<222> (350)..(473)
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<400> 37

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Gly Asp Val Thr Val Ala Pro Ser Asn Phe Ala Asn Gly Val Ala Glu
20 25 30

Trp Ile Ser Ser Asn Ser Arg Ser Gln Ala Tyr Lys Val Thr Cys Ser

35

40

45

Val Arg Gln Ser Ser Ala Gln Lys Arg Lys Tyr Thr Ile Lys Val Glu
50 55 60

Val Pro Lys Val Ala Thr Gln Thr Val Gly Gly Val Glu Leu Pro Val
65 70 75 80

Ala Ala Trp Arg Ser Tyr Leu Asn Met Glu Leu Thr Ile Pro Ile Phe
85 90 95

Ala Thr Asn Ser Asp Cys Glu Leu Ile Val Lys Ala Met Gln Gly Leu
100 105 110

Leu Lys Asp Gly Asn Pro Ile Pro Ser Ala Ile Ala Ala Asn Ser Gly
115 120 125

Ile Tyr Ser Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Pro Lys Lys Lys Arg Lys Val Ala Ala Ala Gly Ser
145 150 155 160

Pro Ser Gly Gln Ile Ser Asn Gln Ala Leu Ala Leu Ala Pro Ser Ser
165 170 175

Ala Pro Val Leu Ala Gln Thr Met Val Pro Ser Ser Ala Met Val Pro
180 185 190

Leu Ala Gln Pro Pro Ala Pro Ala Pro Val Leu Thr Pro Gly Pro Pro
195 200 205

Gln Ser Leu Ser Ala Pro Val Pro Lys Ser Thr Gln Ala Gly Glu Gly
210 215 220

Thr Leu Ser Glu Ala Leu Leu His Leu Gln Phe Asp Ala Asp Glu Asp
225 230 235 240

Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Gly Val Phe Thr Asp
245 250 255

Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln Gly
260 265 270

Val Ser Met Ser His Ser Thr Ala Glu Pro Met Leu Met Glu Tyr Pro
275 280 285

Glu Ala Ile Thr Arg Leu Val Thr Gly Ser Gln Arg Pro Pro Asp Pro
290 295 300

Ala Pro Thr Pro Leu Gly Thr Ser Gly Leu Pro Asn Gly Leu Ser Gly
305 310 315 320

Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala Leu Leu
325 330 335

Ser Gln Ile Ser Ser Ser Gly Gln Gly Gly Gly Gly Ser Gly Phe Ser
340 345 350

Val Asp Thr Ser Ala Leu Leu Asp Leu Phe Ser Pro Ser Val Thr Val
355 360 365

Pro Asp Met Ser Leu Pro Asp Leu Asp Ser Ser Leu Ala Ser Ile Gln
370 375 380

Glu Leu Leu Ser Pro Gln Glu Pro Pro Arg Pro Pro Glu Ala Glu Asn
385 390 395 400

Ser Ser Pro Asp Ser Gly Lys Gln Leu Val His Tyr Thr Ala Gln Pro
405 410 415

Leu Phe Leu Leu Asp Pro Gly Ser Val Asp Thr Gly Ser Asn Asp Leu
420 425 430

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

465

470

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<222> (4228)..(4413)
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tccgacggct tcgccaacag aaacttcatg cagctgatcc acgacgacag cctgaccttt	2160
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