

Sequence Listing

<110> Nanjing GenScript Biotech Co., Ltd.

<120> METHOD FOR CONSTRUCTING A GENE MUTATION LIBRARY

<130> 1

<150> CN201911316464.2

<151> 2019-12-19

<160> 27

<170> PatentIn version 3.5

<210> 1

<211> 40

<212> PRT

<213> Artificial sequence

<220>

<223> Amino acid sequence which requires library construction

<220>

<221> Variant

<222> (8)..(8)

<223> 4 of the 20 amino acids

<220>

<221> Variant

<222> (9)..(9)

<223> 16 of the 20 amino acids

<220>

<221> Variant

<222> (10)..(10)

<223> 16 of the 20 amino acids

<220>

<221> Variant

<222> (12)..(12)

<223> 16 of the 20 amino acids

<220>
<221> Variant
<222> (20)..(20)
<223> 16 of the 20 amino acids

<220>
<221> Variant
<222> (21)..(21)
<223> 16 of the 20 amino acids

<220>
<221> Variant
<222> (33)..(33)
<223> 3 of the 20 amino acids

<400> 1

Arg Pro Asp Val Asn Ala Ser Xaa Xaa Xaa Gly Xaa Thr Pro Leu His
1 5 10 15

Leu Ala Ala Xaa Xaa Gly His Leu Glu Ile Val Glu Val Leu Leu Lys
20 25 30

Xaa Gly Ala Asp Val Arg Pro Asp
35 40

<210> 2
<211> 120
<212> DNA
<213> Artificial sequence

<220>
<223> Nucleotide sequence encoding amino acid sequence 1

<220>
<221> Misc_feature
<222> (22)..(30)
<223> n is a, c, g or t

<220>
<221> Misc_feature
<222> (34)..(36)
<223> n is a, c, g or t

<220>
<221> Misc_feature
<222> (58)..(63)
<223> n is a, c, g or t

<220>
<221> Misc_feature
<222> (97)..(99)
<223> n is a, c, g or t

<400> 2
agacctgacg ttaacgctag cnnnnnnnnn ggtnnnactc cgctgcacct ggctgctnnn 60
nnnggtcacc tggaaatcgt tgaagttctg ctgaagnnng gtgctgacgt gagacctgac 120

<210> 3
<211> 51
<212> DNA
<213> Artificial sequence

<220>
<223> First oligomer

<220>
<221> Misc_feature
<222> (22)..(30)
<223> n is a, c, g or t

<220>
<221> Misc_feature
<222> (34)..(36)
<223> n is a, c, g or t

<400> 3
agacctgacg ttaacgctag cnnnnnnnnn ggtnnnactc cgctgcacct g 51

<210> 4
<211> 6
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse complement sequence of the recognition sequence of BsaI

<400> 4
gagacc 6

<210> 5
<211> 58
<212> DNA
<213> Artificial sequence

<220>
<223> The sequence with the restriction enzyme site introduced

<220>
<221> Misc_feature
<222> (22)..(30)
<223> n is a, c, g or t

<220>
<221> Misc_feature
<222> (34)..(36)
<223> n is a, c, g or t

<400> 5
agacctgacg ttaacgctag cnnnnnnnnn ggtnnnactc cgctgcacct gagagacc 58

<210> 6
<211> 20
<212> DNA
<213> Artificial sequence

<220>

<223> Specific sequence

<400> 6

acctacgtcc aaatcggctg

20

<210> 7

<211> 78

<212> DNA

<213> Artificial sequence

<220>

<223> Oligomer pool-1

<220>

<221> Misc_feature

<222> (22)..(30)

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

<220>

<221> Misc_feature

<222> (34)..(36)

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

<400> 7

agacctgacg ttaacgctag cnnnnnnnnn ggtnnnactc cgctgcacct gagagaccac 60

ctacgtccaa atcggctg

78

<210> 8

<211> 69

<212> DNA

<213> Artificial sequence

<220>

<223> Specific sequence of the second oligomer

<220>

<221> Misc_feature

<222> (7)..(12)

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

<220>

<221> Misc_feature

<222> (46)..(48)

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

<400> 8

gctgctnnnn nnggtcacct ggaaatcggt gaagttctgc tgaagnnngg tgctgacgtg 60

agacctgac 69

<210> 9

<211> 6

<212> DNA

<213> Artificial sequence

<220>

<223> Recognition sequence of restriction endonuclease BsaI

<400> 9

ggtctc 6

<210> 10

<211> 80

<212> DNA

<213> Artificial sequence

<220>

<223> The sequence obtained by additionally adding a base G

<220>

<221> Misc_feature

<222> (18)..(23)

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

<220>

<221> Misc_feature

<222> (57)..(59)

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

<400> 10
ggctctgcct ggctgctnnn nnnnggtcacc tggaaatcgt tgaagttctg ctgaagnnng 60

gtgctgacgt gagacctgac 80

<210> 11
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Specific sequence

<400> 11
actcctaagt aaaatgtgca c 21

<210> 12
<211> 101
<212> DNA
<213> Artificial sequence

<220>
<223> Oligomer pool-2

<220>
<221> Misc_feature
<222> (39)..(44)
<223> n is a, c, g or t

<220>
<221> Misc_feature
<222> (78)..(80)
<223> n is a, c, g or t

<400> 12
actcctaagt aaaatgtgca cggctctgcc tggctgctnn nnnnggtcac ctggaaatcg 60

ttgaagttct gctgaagnnn ggtgctgacg tgagacctga c 101

<210> 13
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> 1F

<400> 13
agacctgacg ttaacgctag 20

<210> 14
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> 1R

<400> 14
cagccgattt ggacgtaggt 20

<210> 15
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> 2F

<400> 15
actcctaagt aaaatgtgca 20

<210> 16
<211> 20
<212> DNA
<213> Artificial sequence

<220>

<223> 2R

<400> 16

gtcaggtctc acgtcagcac

20

<210> 17

<211> 120

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence of assembled oligomer pool

<220>

<221> Misc_feature

<222> (22)..(30)

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

<220>

<221> Misc_feature

<222> (34)..(36)

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

<220>

<221> Misc_feature

<222> (58)..(63)

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

<220>

<221> Misc_feature

<222> (97)..(99)

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

<400> 17

agacctgacg ttaacgctag cnnnnnnnnn ggtnnnactc cgctgcacct ggctgctnnn 60

nnnggtcacc tggaaatcgt tgaagttctg ctgaagnnng gtgctgacgt gagacctgac 120

<210> 18

<211> 32

<212> PRT
<213> Artificial sequence

<220>
<223> Amino acid sequence which requires library construction

<220>
<221> Variant
<222> (14)..(14)
<223> 18 of the 20 amino acids

<220>
<221> Variant
<222> (15)..(15)
<223> 18 of the 20 amino acids

<220>
<221> Variant
<222> (16)..(16)
<223> 18 of the 20 amino acids

<220>
<221> Variant
<222> (17)..(17)
<223> 18 of the 20 amino acids

<220>
<221> Variant
<222> (18)..(18)
<223> 18 of the 20 amino acids

<220>
<221> Variant
<222> (19)..(19)
<223> 18 of the 20 amino acids

<220>
<221> Variant
<222> (19)..(19)
<223> 4 of the 20 amino acids

<400> 18

Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg Asp Xaa Xaa Xaa
 1 5 10 15

Xaa Xaa Xaa Asp Ala Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 20 25 30

<210> 19
 <211> 96
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Nucleotide sequence encoding amino acid sequence 18

<220>
 <221> Misc_feature
 <222> (40)..(57)
 <223> n is a, c, g or t

<400> 19
 aaaaccgagg acacggccgt gtattactgt agtagagatn nnnnnnnnnn nnnnnnngac 60
 gcctggggcc aaggaaccct ggtcaccgtc tcctcg 96

<210> 20
 <211> 51
 <212> DNA
 <213> Artificial sequence

<220>
 <223> First oligomer

<220>
 <221> Misc_feature
 <222> (40)..(51)
 <223> n is a, c, g or t

<400> 20
aaaaccgagg acacggccgt gtattactgt agtagagatn nnnnnnnnnn n 51

<210> 21
<211> 58
<212> DNA
<213> Artificial sequence

<220>
<223> The sequence of the first oligomer with the restriction enzyme site introduced

<220>
<221> Misc_feature
<222> (40)..(51)
<223> n is a, c, g or t

<400> 21
aaaaccgagg acacggccgt gtattactgt agtagagatn nnnnnnnnnn nagagacc 58

<210> 22
<211> 78
<212> DNA
<213> Artificial sequence

<220>
<223> Oligomer pool-1

<220>
<221> Misc_feature
<222> (40)..(51)
<223> n is a, c, g or t

<400> 22
aaaaccgagg acacggccgt gtattactgt agtagagatn nnnnnnnnnn nagagaccac 60

ctacgtccaa atcggctg 78

<210> 23
<211> 51
<212> DNA
<213> Artificial sequence

<220>
<223> Second oligomer

<220>
<221> Misc_feature
<222> (1)..(12)
<223> n is a, c, g or t

<400> 23
nnnnnnnnnn nngacgcctg gggccaagga accctgggtca ccgtctcctc g 51

<210> 24
<211> 56
<212> DNA
<213> Artificial sequence

<220>
<223> The sequence obtained by additionally adding a base G

<220>
<221> Misc_feature
<222> (8)..(17)
<223> n is a, c, g or t

<400> 24
ggctctcgnnn nnnnnnngac gcctggggcc aaggaaccct ggtcacggtc tcctcg 56

<210> 25
<211> 77
<212> DNA
<213> Artificial sequence

<220>
<223> The final sequence of the second oligomer

<220>

<221> Misc_feature

<222> (29)..(38)

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

<400> 25

actcctaagt aaaatgtgca cggctctcgnn nnnnnnnnga cgctggggc caaggaaccc 60

tggtcaccgt ctctcg

77

<210> 26

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> 1sF

<400> 26

aaaaccgagg acacggccgt

20

<210> 27

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> 2sR

<400> 27

cgaggagacg gtgaccagg

20