

9869SG3353-seq1-000001
SEQUENCE LISTING

<110> Agency for Science Technology and Research
Nanyang Technological University

<120> Mutants of The Bacteriophage Lambda Integrase

<130> 9869SG2408

<160> 31

<170> PatentIn version 3.5

<210> 1

<211> 356

<212> PRT

<213> Bacteriophage lambda (Integrase-h/218)

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

Ile Gln Ala Asn Ile Glu Leu Phe Ser Gly His Lys His Lys Pro Leu
50 55 60

Thr Ala Arg Ile Asn Ser Asp Asn Ser Val Thr Leu His Ser Trp Leu
65 70 75 80

Asp Arg Tyr Glu Lys Ile Leu Ala Ser Arg Gly Ile Lys Gln Lys Thr
85 90 95

Leu Ile Asn Tyr Met Ser Lys Ile Lys Ala Ile Arg Arg Gly Leu Pro
100 105 110

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Asp Ala Pro Leu Glu Asp Ile Thr Thr Lys Glu Ile Ala Ala Met Leu
115 120 125

Asn Gly Tyr Ile Asp Glu Gly Lys Ala Ala Ser Ala Lys Leu Ile Arg
130 135 140

Ser Thr Leu Ser Asp Ala Phe Arg Glu Ala Ile Ala Glu Gly His Ile
145 150 155 160

Thr Thr Asn His Val Ala Ala Thr Arg Ala Ala Lys Ser Lys Val Arg
165 170 175

Arg Ser Arg Leu Thr Ala Asp Glu Tyr Leu Lys Ile Tyr Gln Ala Ala
180 185 190

Glu Ser Ser Pro Cys Trp Leu Arg Leu Ala Met Glu Leu Ala Val Val
195 200 205

Thr Gly Gln Arg Val Gly Asp Leu Cys Lys Met Lys Trp Ser Asp Ile
210 215 220

Val Asp Gly Tyr Leu Tyr Val Glu Gln Ser Lys Thr Gly Val Lys Ile
225 230 235 240

Ala Ile Pro Thr Ala Leu His Ile Asp Ala Leu Gly Ile Ser Met Lys
245 250 255

Glu Thr Leu Asp Lys Cys Lys Glu Ile Leu Gly Gly Glu Thr Ile Ile
260 265 270

Ala Ser Thr Arg Arg Glu Pro Leu Ser Ser Gly Thr Val Ser Arg Tyr
275 280 285

Phe Met Arg Ala Arg Lys Ala Ser Gly Leu Ser Phe Glu Gly Asp Pro
290 295 300

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Pro Thr Phe His Glu Leu Arg Ser Leu Ser Ala Arg Leu Tyr Glu Lys
305 310 315 320

Gln Ile Ser Asp Lys Phe Ala Gln His Leu Leu Gly His Lys Ser Asp
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Thr Met Ala Ser Gln Tyr Arg Asp Asp Arg Gly Arg Glu Trp Asp Lys
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Ile Glu Ile Lys
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<212> PRT

<213> artificial

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<223> C2 lambda integrase mutant

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Met Gly Arg Arg Arg Ser His Glu Arg Arg Asp Leu Pro Pro Asn Leu
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Tyr Ile Arg Asn Asn Gly Tyr Tyr Cys Tyr Arg Asp Pro Arg Thr Gly
20 25 30

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

Ile Gln Ala Asn Ile Glu Leu Phe Ser Gly His Lys His Lys Pro Leu
50 55 60

Thr Ala Arg Ile Asn Ser Asp Asn Ser Val Thr Leu His Ser Trp Leu
65 70 75 80

Asp Arg Tyr Glu Lys Ile Leu Ala Ser Arg Gly Ile Lys Gln Lys Thr
85 90 95

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Leu Ile Asn Tyr Met Ser Lys Ile Lys Ala Ile Arg Arg Gly Leu Pro
100 105 110

Asp Ala Pro Leu Glu Asp Ile Thr Thr Lys Glu Ile Ala Ala Met Leu
115 120 125

Asn Gly Tyr Ile Asp Glu Gly Lys Ala Ala Ser Ala Lys Leu Ile Arg
130 135 140

Ser Thr Leu Ser Asp Ala Phe Arg Glu Ala Ile Ala Glu Gly His Ile
145 150 155 160

Thr Thr Asn His Val Ala Ala Thr Arg Ala Ala Lys Ser Lys Val Arg
165 170 175

Arg Ser Arg Leu Thr Ala Asp Glu Tyr Leu Lys Ile Tyr Gln Ala Ala
180 185 190

Glu Ser Ser Pro Cys Trp Leu Arg Leu Ala Met Glu Leu Ala Val Val
195 200 205

Thr Gly Gln Arg Val Gly Asp Leu Cys Lys Met Lys Trp Ser Asp Ile
210 215 220

Val Asp Gly Tyr Leu Tyr Val Glu Gln Ser Lys Thr Gly Val Lys Ile
225 230 235 240

Ala Ile Pro Thr Ala Leu His Ile Asp Ala Leu Gly Ile Ser Met Lys
245 250 255

Glu Thr Leu Asp Lys Cys Lys Glu Ile Leu Gly Gly Glu Thr Ile Ile
260 265 270

Ala Ser Thr Arg Arg Glu Pro Leu Ser Ser Gly Thr Val Ser Arg Tyr
275 280 285

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Phe Met Arg Ala Arg Lys Ala Ser Gly Leu Ser Phe Glu Gly Asp Pro
290 295 300

Pro Thr Phe His Glu Leu Arg Ser Leu Ser Ala Arg Leu Tyr Glu Lys
305 310 315 320

Gln Ile Ser Asp Lys Phe Ala Gln His Leu Leu Gly His Lys Ser Val
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Thr Met Ala Ser Gln Tyr Arg Asp Asp Arg Gly Arg Glu Trp Asp Lys
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Ile Glu Ile Lys
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<212> PRT
<213> artificial

<220>
<223> C3 lambda integrase mutant

<400> 3

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

Ile Gln Ala Asn Ile Glu Leu Phe Ser Gly His Lys His Lys Pro Leu
50 55 60

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Thr Ala Arg Ile Asn Ser Asp Asn Ser Val Thr Leu His Ser Trp Leu
65 70 75 80

Asp Arg Tyr Glu Lys Ile Leu Ala Ser Arg Gly Ile Lys Gln Lys Thr
85 90 95

Leu Ile Asn Tyr Met Ser Lys Ile Lys Ala Ile Arg Arg Gly Leu Pro
100 105 110

Asp Ala Pro Leu Glu Asp Ile Thr Thr Lys Glu Ile Ala Ala Met Leu
115 120 125

Asn Gly Tyr Ile Asp Glu Gly Lys Ala Ala Ser Ala Lys Leu Ile Arg
130 135 140

Ser Thr Leu Ser Asp Ala Phe Arg Glu Ala Ile Ala Glu Gly His Ile
145 150 155 160

Thr Thr Asn His Val Ala Ala Thr Arg Ala Ala Lys Ser Lys Val Arg
165 170 175

Arg Ser Arg Leu Thr Ala Asp Glu Tyr Leu Lys Ile Tyr Gln Ala Ala
180 185 190

Glu Ser Ser Pro Cys Trp Leu Arg Leu Ala Met Glu Leu Ala Val Val
195 200 205

Thr Gly Gln Arg Val Gly Asp Leu Cys Lys Met Lys Trp Ser Asp Ile
210 215 220

Val Asp Gly Tyr Leu Tyr Val Glu Gln Ser Lys Thr Gly Val Lys Ile
225 230 235 240

Ala Ile Pro Thr Ala Leu His Ile Asp Ala Leu Gly Ile Ser Met Lys
245 250 255

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Glu Thr Leu Asp Lys Cys Lys Glu Ile Leu Gly Gly Glu Thr Ile Ile
260 265 270

Ala Ser Thr Arg Arg Glu Pro Leu Ser Ser Gly Thr Val Ser Arg Tyr
275 280 285

Phe Met Arg Ala Arg Lys Ala Ser Gly Leu Ser Phe Glu Gly Asp Pro
290 295 300

Pro Thr Phe His Glu Leu Arg Ser Leu Ser Ala Arg Leu Tyr Gly Lys
305 310 315 320

Gln Ile Ser Asp Lys Phe Ala Gln His Leu Leu Gly His Lys Ser Val
325 330 335

Thr Met Ala Ser Gln Tyr Arg Asp Asp Arg Gly Arg Glu Trp Asp Lys
340 345 350

Ile Glu Ile Lys
355

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gatcgctacg aaaaaatcct ggccagcaga ggaatcaagc agaagacact cataaattac 300
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gaaggggatc cgcctacctt tcacgagttg cgcagtttgt ctgcaagact ctatgagaag	960
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<223> EcoliAttB-F primer

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<211> 21

<212> DNA

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<223> EcoliAttB-R primer

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<210> 18

<211> 1071

<212> DNA

<213> Artificial Sequence

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<223> C2 mutant gene

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aggcgaatcg caatcactga agctatacag gccaacattg agttattttc aggacacaaa 180

cacaagcctc tgacagcgag aatcaacagt gataattccg ttacgttaca ttcattggctt 240

gatcgctacg aaaaaatcct ggccagcaga ggaatcaagc agaagacact cataaattac 300

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acaaaagaaa ttgcggcaat gctcaatgga tacatagacg agggcaaggc ggcgtcagcc 420

aagttaatca gatcaacgct gagcgatgca ttccgagagg caatagctga aggccatata 480

acaacaaacc atgtcgctgc cactcgcgca gcaaagtcaa aggttaaggag atcaagactt 540

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cagataagcg ataagtttgc tcaacatctt ctcgggcata agtcgggtcac catggcatca	1020
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 <213> Artificial Sequence

<220>
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aggcgattcg caatcactga agctatacag gccaacattg agttatcttc aggacacaaa	180
cacaagcctc tgacagcgag aatcaacagt gataattccg ttacgttaca ttcatggctt	240
gatcgctacg aaaaaatcct ggccagcaga ggaatcaagc agaagacact cataaattac	300
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tcatccggca cagtatcaag gtattttatg cgcgcacgaa aagcatcagg tctttccttc	900
gaaggggatc cgcctacctt tcacgagttg cgcagtttgt ctgcaagact ctatgggaag	960
cagataagcg ataagtttgc tcaacatctt ctcgggcata agtcgggtcac catggcatca	1020
cagtatcgtg atgacagagg cagggagtgg gacaaaattg aaatcaaata a	1071

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<212> DNA

<213> Artificial Sequence

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<223> C3 mutant minicircle DNA

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tataggggaa ttgtgagcgg ataacaattc ccctctagaa ataattttgt ttaactttaa	180
gaaggagata tacatatggg aagaaggcga agtcatgagc gccgggattt accccctaac	240
ctttatataa gaaacaatgg atattactgc tacagggacc caaggacggg taaagagttt	300
ggattaggca gagacaggcg attcgcaatc actgaagcta tacaggccaa cattgagtta	360
ttttcaggac acaaacacaa gcctctgaca gcgagaatca acagtgataa ttccgttacg	420
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taaaaaagca ttgcttatca atttgttgca acgaac	caggt cactatcagt caaaataaaa	240
tcattatttg atttcccggt gatgttcagg tggcact	tttt cggggaaatg tgcgcggaac	300
ccctatttgt ttatttttct aaatacattc aaatat	gtat ccgctcatga gacaataacc	360

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ctgataaatg cttcaataat attgaaaaag gaagagtatg agtattcaac atttccgtgt	420
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ggtgaaagta aaagatgctg aagatcagtt ggggtgcacga gtgggttaca tcgaactgga	540
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cacttttaaa gttctgctat gtggcgcggt attatcccgt attgacgccg ggcaagagca	660
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<400> 22
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<210> 23
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<212> DNA
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<400> 23
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<211> 331
<212> DNA
<213> Artificial Sequence

<220>
<223> PGK promoter

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<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> HOP'attH4X_F1 primer

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<210> 26
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> PuroRev24 primer

<400> 26
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<210> 27
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> pLIR-F1 primer

<400> 27
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<210> 28
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> pLIR-R1 primer

<400> 28
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<210> 29
 <211> 4906
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> pPGKssPuro-attP4X (targeting vector)

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aagcattgct tatcaatttg ttgcaacgaa caggtcacta tcagtcaaaa taaaatcatt	240
atttgatttc aattttgtcc cactccctcc cgaattctac cgggtagggg aggcgctttt	300
cccaaggcag tctggagcat gcgctttagc agccccgctg gcacttggcg ctacacaagt	360
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