

# SEQUENCE LISTING

<110> Commonwealth Scientific and Industrial Research Organisation

<120> Sensor for detecting hydrolysis

<130> 524960PCT

<150> AU2017903420

<151> 2017-08-24

<160> 51

<170> PatentIn version 3.5

<210> 1

<211> 349

<212> PRT

<213> Artificial Sequence

<220>

<223> wt-RLuc8

<400> 1

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe Met Ala Ser Lys Val Tyr Asp Pro Glu Gln  
35 40 45

Arg Lys Arg Met Ile Thr Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln  
50 55 60

Met Asn Val Leu Asp Ser Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His  
65 70 75 80

Ala Glu Asn Ala Val Ile Phe Leu His Gly Asn Ala Thr Ser Ser Tyr  
85 90 95

Leu Trp Arg His Val Val Pro His Ile Glu Pro Val Ala Arg Cys Ile  
100 105 110

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

Ser Tyr Arg Leu Leu Asp His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu  
130 135 140

Leu Leu Asn Leu Pro Lys Lys Ile Ile Phe Val Gly His Asp Trp Gly  
145 150 155 160

Ala Ala Leu Ala Phe His Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys  
165 170 175

Ala Ile Val His Met Glu Ser Val Val Asp Val Ile Glu Ser Trp Asp  
180 185 190

Glu Trp Pro Asp Ile Glu Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu  
195 200 205

Gly Glu Lys Met Val Leu Glu Asn Asn Phe Phe Val Glu Thr Val Leu  
210 215 220

Pro Ser Lys Ile Met Arg Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr  
225 230 235 240

Leu Glu Pro Phe Lys Glu Lys Gly Glu Val Arg Arg Pro Thr Leu Ser  
245 250 255

Trp Pro Arg Glu Ile Pro Leu Val Lys Gly Gly Lys Pro Asp Val Val  
260 265 270

Gln Ile Val Arg Asn Tyr Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu  
275 280 285

Pro Lys Leu Phe Ile Glu Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile  
290 295 300

Val Glu Gly Ala Lys Lys Phe Pro Asn Thr Glu Phe Val Lys Val Lys  
305 310 315 320

Gly Leu His Phe Leu Gln Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr  
325 330 335

Ile Lys Ser Phe Val Glu Arg Val Leu Lys Asn Glu Gln  
340 345

<210> 2  
<211> 349  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> RLuc8Cys1

<400> 2

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Cys Met Ala Ser Lys Val Tyr Asp Pro Glu Gln  
35 40 45

Arg Lys Arg Met Ile Thr Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln  
50 55 60

Met Asn Val Leu Asp Ser Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His  
65 70 75 80

Ala Glu Asn Ala Val Ile Phe Leu His Gly Asn Ala Thr Ser Ser Tyr  
85 90 95

Leu Trp Arg His Val Val Pro His Ile Glu Pro Val Ala Arg Cys Ile  
100 105 110

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

Ser Tyr Arg Leu Leu Asp His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu  
130 135 140

Leu Leu Asn Leu Pro Lys Lys Ile Ile Phe Val Gly His Asp Trp Gly  
145 150 155 160

Ala Ala Leu Ala Phe His Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys  
165 170 175

Ala Ile Val His Met Glu Ser Val Val Asp Val Ile Glu Ser Trp Asp  
180 185 190

Glu Trp Pro Asp Ile Glu Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu  
195 200 205

Gly Glu Lys Met Val Leu Glu Asn Asn Phe Phe Val Glu Thr Val Leu  
210 215 220

Pro Ser Lys Ile Met Arg Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr  
225 230 235 240

Leu Glu Pro Phe Lys Glu Lys Gly Glu Val Arg Arg Pro Thr Leu Ser  
245 250 255

Trp Pro Arg Glu Ile Pro Leu Val Lys Gly Gly Lys Pro Asp Val Val  
260 265 270

Gln Ile Val Arg Asn Tyr Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu  
275 280 285

Pro Lys Leu Phe Ile Glu Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile  
290 295 300

Val Glu Gly Ala Lys Lys Phe Pro Asn Thr Glu Phe Val Lys Val Lys  
305 310 315 320

Gly Leu His Phe Leu Gln Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr  
325 330 335

Ile Lys Ser Phe Val Glu Arg Val Leu Lys Asn Glu Gln  
340 345

<210> 3  
<211> 349  
<212> PRT  
<213> Artificial Sequence

<220>

<223> RLuc8Cys2

<400> 3

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Cys Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe Met Ala Ser Lys Val Tyr Asp Pro Glu Gln  
35 40 45

Arg Lys Arg Met Ile Thr Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln  
50 55 60

Met Asn Val Leu Asp Ser Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His  
65 70 75 80

Ala Glu Asn Ala Val Ile Phe Leu His Gly Asn Ala Thr Ser Ser Tyr  
85 90 95

Leu Trp Arg His Val Val Pro His Ile Glu Pro Val Ala Arg Cys Ile  
100 105 110

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

Ser Tyr Arg Leu Leu Asp His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu  
130 135 140

Leu Leu Asn Leu Pro Lys Lys Ile Ile Phe Val Gly His Asp Trp Gly  
145 150 155 160

Ala Ala Leu Ala Phe His Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys  
165 170 175

Ala Ile Val His Met Glu Ser Val Val Asp Val Ile Glu Ser Trp Asp  
180 185 190

Glu Trp Pro Asp Ile Glu Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu  
195 200 205

Gly Glu Lys Met Val Leu Glu Asn Asn Phe Phe Val Glu Thr Val Leu  
210 215 220

Pro Ser Lys Ile Met Arg Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr  
225 230 235 240

Leu Glu Pro Phe Lys Glu Lys Gly Glu Val Arg Arg Pro Thr Leu Ser  
245 250 255

Trp Pro Arg Glu Ile Pro Leu Val Lys Gly Gly Lys Pro Asp Val Val  
260 265 270

Gln Ile Val Arg Asn Tyr Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu  
275 280 285

Pro Lys Leu Phe Ile Glu Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile  
290 295 300

Val Glu Gly Ala Lys Lys Phe Pro Asn Thr Glu Phe Val Lys Val Lys  
305 310 315 320

Gly Leu His Phe Leu Gln Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr  
325 330 335

Ile Lys Ser Phe Val Glu Arg Val Leu Lys Asn Glu Gln  
340 345

<210> 4

<211> 349

<212> PRT

<213> Artificial Sequence

<220>

<223> RLuc8Cys3

<400> 4

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Cys Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe Met Ala Ser Lys Val Tyr Asp Pro Glu Gln  
35 40 45

Arg Lys Arg Met Ile Thr Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln  
50 55 60

Met Asn Val Leu Asp Ser Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His  
65 70 75 80

Ala Glu Asn Ala Val Ile Phe Leu His Gly Asn Ala Thr Ser Ser Tyr  
85 90 95

Leu Trp Arg His Val Val Pro His Ile Glu Pro Val Ala Arg Cys Ile  
100 105 110

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

Ser Tyr Arg Leu Leu Asp His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu  
130 135 140

Leu Leu Asn Leu Pro Lys Lys Ile Ile Phe Val Gly His Asp Trp Gly  
145 150 155 160

Ala Ala Leu Ala Phe His Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys  
165 170 175

Ala Ile Val His Met Glu Ser Val Val Asp Val Ile Glu Ser Trp Asp  
180 185 190

Glu Trp Pro Asp Ile Glu Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu  
195 200 205

Gly Glu Lys Met Val Leu Glu Asn Asn Phe Phe Val Glu Thr Val Leu  
210 215 220

Pro Ser Lys Ile Met Arg Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr  
225 230 235 240

Leu Glu Pro Phe Lys Glu Lys Gly Glu Val Arg Arg Pro Thr Leu Ser  
245 250 255

Trp Pro Arg Glu Ile Pro Leu Val Lys Gly Gly Lys Pro Asp Val Val  
260 265 270

Gln Ile Val Arg Asn Tyr Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu  
275 280 285

Pro Lys Leu Phe Ile Glu Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile  
290 295 300

Val Glu Gly Ala Lys Lys Phe Pro Asn Thr Glu Phe Val Lys Val Lys  
305 310 315 320

Gly Leu His Phe Leu Gln Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr  
325 330 335

Ile Lys Ser Phe Val Glu Arg Val Leu Lys Asn Glu Gln  
340 345

<210> 5  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linking element

<400> 5

Cys Asp Asp Lys Asp Arg Trp Gly Ser Glu Phe  
1 5 10

<210> 6  
<211> 21  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linking element

<400> 6

Cys Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp Arg  
1 5 10 15

Trp Gly Ser Glu Phe  
20

<210> 7  
<211> 38  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linking element

<400> 7

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe  
35

<210> 8  
<211> 311  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> wt-RLuc8(C24X)

<220>  
<221> X  
<222> (24)..(24)  
<223> X is any amino acid other than a cysteine.

<400> 8

Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
1 5 10 15

Gly Pro Gln Trp Trp Ala Arg Xaa Lys Gln Met Asn Val Leu Asp Ser  
20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
35 40 45

Phe Leu His Gly Asn Ala Thr Ser Ser Tyr Leu Trp Arg His Val Val  
50 55 60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly  
65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His  
115 120 125

Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys Ala Ile Val His Met Glu  
130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
145 150 155 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
165 170 175

Glu Asn Asn Phe Phe Val Glu Thr Val Leu Pro Ser Lys Ile Met Arg  
180 185 190

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
195 200 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
210 215 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
225 230 235 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Leu Phe Ile Glu  
245 250 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
260 265 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Leu Gln  
275 280 285

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
290 295 300

Arg Val Leu Lys Asn Glu Gln  
305 310

<210> 9  
<211> 311  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> wt-RLuc8(C73Z)

<220>  
<221> Z  
<222> (73)..(73)  
<223> Z is any amino acid other than a cysteine.

<400> 9

Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
1 5 10 15

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
35 40 45

Phe Leu His Gly Asn Ala Thr Ser Ser Tyr Leu Trp Arg His Val Val  
50 55 60

Pro His Ile Glu Pro Val Ala Arg Glx Ile Ile Pro Asp Leu Ile Gly  
65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His  
115 120 125

Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys Ala Ile Val His Met Glu  
130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
145 150 155 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
165 170 175

Glu Asn Asn Phe Phe Val Glu Thr Val Leu Pro Ser Lys Ile Met Arg  
180 185 190

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
195 200 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
210 215 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
225 230 235 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Leu Phe Ile Glu  
245 250 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
260 265 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Leu Gln  
275 280 285

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
290 295 300

Arg Val Leu Lys Asn Glu Gln  
305 310

<210> 10  
<211> 311  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> wt-RLuc8(C24X.C73Z)

<220>  
<221> X  
<222> (24)..(24)  
<223> X is any amino acid other than a cysteine.

<220>  
<221> Z  
<222> (73)..(73)  
<223> Z is any amino acid other than a cysteine.

<400> 10

Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
1 5 10 15

Gly Pro Gln Trp Trp Ala Arg Xaa Lys Gln Met Asn Val Leu Asp Ser  
20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
35 40 45

Phe Leu His Gly Asn Ala Thr Ser Ser Tyr Leu Trp Arg His Val Val  
50 55 60

Pro His Ile Glu Pro Val Ala Arg Glx Ile Ile Pro Asp Leu Ile Gly  
65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His  
115 120 125

Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys Ala Ile Val His Met Glu  
130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
145 150 155 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
165 170 175

Glu Asn Asn Phe Phe Val Glu Thr Val Leu Pro Ser Lys Ile Met Arg  
180 185 190

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
195 200 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
210 215 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
225 230 235 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Leu Phe Ile Glu  
245 250 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
260 265 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Leu Gln  
275 280 285

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
290 295 300

Arg Val Leu Lys Asn Glu Gln  
305 310

<211> 1050  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> nucleotide sequence encoding wt-RLuc8

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atgggtcggg atctgtacga cgatgacgat aaggatcgat ggggatccga attcatggct 120  
tccaaggtgt acgaccccga gcaacgcaaa cgcatgatca ctgggcctca gtggtgggct 180  
cgctgcaagc aaatgaacgt gctggactcc ttcatcaact actatgattc cgagaagcac 240  
gccgagaacg ccgtgatttt tctgcatggg aacgctacct ccagctacct gtggaggcac 300  
gtcgtgcctc acatcgagcc cgtggctaga tgcacatcc ctgatctgat cggaatgggt 360  
aagtccggca agagcgggaa tggctcatat cgcctcctgg atcactaaa gtacctcacc 420  
gcttggttcg agctgctgaa ctttccaaag aaaatcatct ttgtgggcca cgactggggg 480  
gctgctctgg cttttcacta cgcctacgag caccaagaca ggatcaaggc catcgtccat 540  
atggagagtg tcgtggacgt gatcgagtcc tgggacgagt ggcttgacat cgaggaggat 600  
atgccctga tcaagagcga agagggcgag aaaatgggtgc ttgagaataa cttcttcgtc 660  
gagaccgtgc tccaagcaa gatcatgcgg aaactggagc ctgaggagtt cgctgcctac 720  
ctggagccat tcaaggagaa gggcgagggt agacggccta ccctctcctg gcctcgcgag 780  
atccctctcg ttaagggagg caagcccgac gtcgtccaga ttgtccgcaa ctacaacgcc 840  
taccttcggg ccagcgacga tctgcctaag ctgttcacat agtccgaccc tgggttcttt 900  
tccaacgcta ttgtcgaggg agctaagaag ttccctaaca ccgagttcgt gaaggtgaag 960  
ggcctccact tcctccagga ggacgctcca gatgaaatgg gtaagtacat caagagcttc 1020  
gtggagcgcg tgctgaagaa cgagcagtaa 1050

<210> 12  
<211> 1050  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> nucleotide sequence encoding RLuc8Cys1

<400> 12

|                                                                    |      |
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| atgggtcggg atctgtacga cgatgacgat aaggatcgat ggggatccga atgcatggct  | 120  |
| tccaaggtgt acgaccccg gcaacgcaaa cgcatgatca ctgggcctca gtggtgggct   | 180  |
| cgctgcaagc aaatgaacgt gctggactcc ttcatcaact actatgattc cgagaagcac  | 240  |
| gccgagaacg ccgtgatttt tctgcatggg aacgctacct ccagctacct gtggaggcac  | 300  |
| gtcgtgcctc acatcgagcc cgtggctaga tgcacatcc ctgatctgat cggaatgggt   | 360  |
| aagtccggca agagcgggaa tggctcatat cgcctcctgg atcactacaa gtacctcacc  | 420  |
| gcttggttcg agctgctgaa ccttccaaag aaaatcatct ttgtgggcca cgactggggg  | 480  |
| gctgctctgg cttttcacta cgcctacgag caccaagaca ggatcaaggc catcgtccat  | 540  |
| atggagagtg tcgtggacgt gatcgagtcc tgggacgagt ggcctgacat cgaggaggat  | 600  |
| atgccctga tcaagagcga agagggcgag aaaatggtgc ttgagaataa cttcttcgtc   | 660  |
| gagaccgtgc tccaagcaa gatcatgcgg aaactggagc ctgaggagtt cgctgcctac   | 720  |
| ctggagccat tcaaggagaa gggcgagggt agacggccta ccctctcctg gcctcgcgag  | 780  |
| atccctctcg ttaagggagg caagcccgac gtcgtccaga ttgtccgcaa ctacaacgcc  | 840  |
| taccttcggg ccagcgacga tctgcctaag ctgttcacat agtccgaccc tgggttcttt  | 900  |
| tccaacgcta ttgtcgaggg agctaagaag ttccctaaca ccgagttcgt gaaggtgaag  | 960  |
| ggcctccact tcctccagga ggacgctcca gatgaaatgg gtaagtacat caagagcttc  | 1020 |
| gtggagcgcg tgctgaagaa cgagcagtaa                                   | 1050 |

<210> 13  
 <211> 1050  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> nucleotide sequence encoding RLuc8Cys2

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| <400> 13                                                           |     |
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| atgggtcggg atctgtacga ctgcgacgat aaggatcgat ggggatccga attcatggct  | 120 |
| tccaaggtgt acgaccccg gcaacgcaaa cgcatgatca ctgggcctca gtggtgggct   | 180 |
| cgctgcaagc aaatgaacgt gctggactcc ttcatcaact actatgattc cgagaagcac  | 240 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| gccgagaacg ccgtgatttt tctgcatggt aacgctacct ccagctacct gtggaggcac | 300  |
| gtcgtgcctc acatcgagcc cgtggctaga tgcacatcc ctgatctgat cggaatgggt  | 360  |
| aagtccggca agagcgggaa tggctcatat cgcctcctgg atcactacaa gtacctcacc | 420  |
| gcttggttcg agctgctgaa ctttccaaag aaaatcatct ttgtgggcca cgactggggg | 480  |
| gctgctctgg cttttcacta cgcctacgag caccaagaca ggatcaaggc catcgtccat | 540  |
| atggagagtg tcgtggacgt gatcgagtcc tgggacgagt ggcttgacat cgaggaggat | 600  |
| atcgccctga tcaagagcga agagggcgag aaaatggtgc ttgagaataa cttcttcgtc | 660  |
| gagaccgtgc tccaagcaa gatcatgcgg aaactggagc ctgaggagtt cgctgcctac  | 720  |
| ctggagccat tcaaggagaa gggcgagggt agacggccta ccctctcctg gcctcgcgag | 780  |
| atccctctcg ttaagggagg caagcccgac gtcgtccaga ttgtccgcaa ctacaacgcc | 840  |
| taccttcggg ccagcgacga tctgcctaag ctgttcacg agtccgacc tgggttcttt   | 900  |
| tccaacgcta ttgtcgaggg agctaagaag ttccctaaca ccgagttcgt gaaggtgaag | 960  |
| ggcctccact tcctccagga ggacgtcca gatgaaatgg gtaagtacat caagagcttc  | 1020 |
| gtggagcgcg tgctgaagaa cgagcagtaa                                  | 1050 |

<210> 14

<211> 1050

<212> DNA

<213> Artificial Sequence

<220>

<223> nucleotide sequence encoding RLuc8Cys3

<400> 14

|                                                                    |     |
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| atgggtcggg atctgtacga cgatgacgat aaggatcgat ggggatccga attcatggct  | 120 |
| tccaaggtgt acgaccccga gcaacgcaa cgcacgatca ctgggcctca gtggtgggct   | 180 |
| cgctgcaagc aaatgaacgt gctggactcc ttcacaaact actatgattc cgagaagcac  | 240 |
| gccgagaacg ccgtgatttt tctgcatggt aacgctacct ccagctacct gtggaggcac  | 300 |
| gtcgtgcctc acatcgagcc cgtggctaga tgcacatcc ctgatctgat cggaatgggt   | 360 |
| aagtccggca agagcgggaa tggctcatat cgcctcctgg atcactacaa gtacctcacc  | 420 |
| gcttggttcg agctgctgaa ctttccaaag aaaatcatct ttgtgggcca cgactggggg  | 480 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| gctgctctgg cctttcacta cgcctacgag caccaagaca ggatcaaggc catcgtccat | 540  |
| atggagagtg tcgtggacgt gatcgagtcc tgggacgagt ggcctgacat cgaggaggat | 600  |
| atcgccctga tcaagagcga agagggcgag aaaatggtgc ttgagaataa cttcttcgtc | 660  |
| gagaccgtgc tccaagcaa gatcatgcgg aaactggagc ctgaggagtt cgctgcctac  | 720  |
| ctggagccat tcaaggagaa gggcgaggtt agacggccta ccctctcctg gcctcgcgag | 780  |
| atccctctcg ttaagggagg caagcccgac gtcgtccaga ttgtccgcaa ctacaacgcc | 840  |
| taccttcggg ccagcgacga tctgcctaag ctgttcacg agtccgaccc tgggttcttt  | 900  |
| tccaacgcta ttgtcgaggg agctaagaag ttccctaaca ccgagttcgt gaaggtgaag | 960  |
| ggcctccact tcctccagga ggacgtcca gatgaaatgg gtaagtacat caagagcttc  | 1020 |
| gtggagcgcg tgctgaagaa cgagcagtaa                                  | 1050 |

<210> 15  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RLuc8Cys1-F

|                                 |    |
|---------------------------------|----|
| <400> 15                        |    |
| atggggatcc gaatgcatgg cttccaagg | 29 |

<210> 16  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RLuc8Cys1-R

|                                |    |
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| <400> 16                       |    |
| ccttggaagc catgcattcg gatcccat | 29 |

<210> 17  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RLuc8Cys2-F

<400> 17

ggatctgtac gactgcgacg ataaggatcg 30

<210> 18  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RLuc8Cys2-R

<400> 18  
cgatccttat cgtcgagtc gtacagatcc 30

<210> 19  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RLuc8Cys3-F

<400> 19  
ctagcatgac tggttgccag caaatgggtc 30

<210> 20  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> RLuc8Cys3-R

<400> 20  
gaccatttg ctggcaacca gtcattgtag 30

<210> 21  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> High affinity substrate for mTG

<400> 21

Trp Ala Leu Gln Arg Pro His  
1 5

<210> 22

<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> High affinity substrate for mTG

<400> 22

Trp Glu Leu Gln Arg Pro Tyr  
1 5

<210> 23  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Sortase recognition sequence

<220>  
<221> X  
<222> (3)..(3)  
<223> X is any amino acid

<400> 23

Leu Pro Xaa Thr  
1

<210> 24  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> spacer sequence

<400> 24

Gly Ser Ser Gly Gly Ser  
1 5

<210> 25  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Spacer sequence

<400> 25

Gly Gly Ser Gly Gly Ser  
1 5

<210> 26

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Spacer sequence

<400> 26

Gly Gly Thr Gly Gly Gly  
1 5

<210> 27

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Spacer sequence

<400> 27

Gly Gly Gly Gly Gly Thr  
1 5

<210> 28

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Spacer sequence

<400> 28

Leu Gln Gly Gly Thr Gly Gly Gly  
1 5

<210> 29

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Specaer sequence

<400> 29

Phe Glu Gly Gly Thr Gly Gly Gly  
1 5

<210> 30

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Spacer sequence

<400> 30

Gly Gly Ser Gly Gly Ser Leu  
1 5

<210> 31

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity substrate for mTG

<220>

<221> X

<222> (3)..(3)

<223> X is any amino acid

<220>

<221> Z

<222> (5)..(5)

<223> Z is Gly or Ala

<400> 31

Leu Pro Xaa Thr Glx  
1 5

<210> 32

<211> 349

<212> PRT

<213> Artificial Sequence

<220>

<223> RLuc8Cys4

<400> 32

Met Arg Gly Ser His His His His His His Cys Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe Met Ala Ser Lys Val Tyr Asp Pro Glu Gln  
35 40 45

Arg Lys Arg Met Ile Thr Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln  
50 55 60

Met Asn Val Leu Asp Ser Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His  
65 70 75 80

Ala Glu Asn Ala Val Ile Phe Leu His Gly Asn Ala Thr Ser Ser Tyr  
85 90 95

Leu Trp Arg His Val Val Pro His Ile Glu Pro Val Ala Arg Cys Ile  
100 105 110

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

Ser Tyr Arg Leu Leu Asp His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu  
130 135 140

Leu Leu Asn Leu Pro Lys Lys Ile Ile Phe Val Gly His Asp Trp Gly  
145 150 155 160

Ala Ala Leu Ala Phe His Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys  
165 170 175

Ala Ile Val His Met Glu Ser Val Val Asp Val Ile Glu Ser Trp Asp  
180 185 190

Glu Trp Pro Asp Ile Glu Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu  
195 200 205

Gly Glu Lys Met Val Leu Glu Asn Asn Phe Phe Val Glu Thr Val Leu  
210 215 220

Pro Ser Lys Ile Met Arg Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr  
225 230 235 240

Leu Glu Pro Phe Lys Glu Lys Gly Glu Val Arg Arg Pro Thr Leu Ser  
245 250 255

Trp Pro Arg Glu Ile Pro Leu Val Lys Gly Gly Lys Pro Asp Val Val  
260 265 270

Gln Ile Val Arg Asn Tyr Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu  
275 280 285

Pro Lys Leu Phe Ile Glu Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile  
290 295 300

Val Glu Gly Ala Lys Lys Phe Pro Asn Thr Glu Phe Val Lys Val Lys  
305 310 315 320

Gly Leu His Phe Leu Gln Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr  
325 330 335

Ile Lys Ser Phe Val Glu Arg Val Leu Lys Asn Glu Gln  
340 345

<210> 33  
<211> 349  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> RLuc8Cys5

<400> 33

Met Cys Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe Met Ala Ser Lys Val Tyr Asp Pro Glu Gln  
35 40 45

Arg Lys Arg Met Ile Thr Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln  
50 55 60

Met Asn Val Leu Asp Ser Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His  
65 70 75 80

Ala Glu Asn Ala Val Ile Phe Leu His Gly Asn Ala Thr Ser Ser Tyr  
85 90 95

Leu Trp Arg His Val Val Pro His Ile Glu Pro Val Ala Arg Cys Ile  
100 105 110

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

Ser Tyr Arg Leu Leu Asp His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu  
130 135 140

Leu Leu Asn Leu Pro Lys Lys Ile Ile Phe Val Gly His Asp Trp Gly  
145 150 155 160

Ala Ala Leu Ala Phe His Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys  
165 170 175

Ala Ile Val His Met Glu Ser Val Val Asp Val Ile Glu Ser Trp Asp  
180 185 190

Glu Trp Pro Asp Ile Glu Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu  
195 200 205

Gly Glu Lys Met Val Leu Glu Asn Asn Phe Phe Val Glu Thr Val Leu  
210 215 220

Pro Ser Lys Ile Met Arg Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr  
225 230 235 240

Leu Glu Pro Phe Lys Glu Lys Gly Glu Val Arg Arg Pro Thr Leu Ser  
245 250 255

Trp Pro Arg Glu Ile Pro Leu Val Lys Gly Gly Lys Pro Asp Val Val  
260 265 270

Gln Ile Val Arg Asn Tyr Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu  
275 280 285

Pro Lys Leu Phe Ile Glu Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile  
290 295 300

Val Glu Gly Ala Lys Lys Phe Pro Asn Thr Glu Phe Val Lys Val Lys  
305 310 315 320

Gly Leu His Phe Leu Gln Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr  
325 330 335

Ile Lys Ser Phe Val Glu Arg Val Leu Lys Asn Glu Gln  
340 345

<210> 34  
<211> 724  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> MBP(K239C)RLuc8

<400> 34

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe Met Lys Ile Glu Glu Gly Lys Leu Val Ile  
35 40 45

Trp Ile Asn Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys  
50 55 60

Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp  
65 70 75 80

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

Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser  
100 105 110

Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu  
115 120 125

Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala  
130 135 140

Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu  
145 150 155 160

Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys  
165 170 175

Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu  
180 185 190

Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe  
195 200 205

Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn  
210 215 220

Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn  
225 230 235 240

Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe  
245 250 255

Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser  
260 265 270

Asn Ile Asp Thr Ser Cys Val Asn Tyr Gly Val Thr Val Leu Pro Thr  
275 280 285

Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly  
290 295 300

Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu  
305 310 315 320

Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys  
325 330 335

Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Val Lys  
340 345 350

Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile  
355 360 365

Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr  
370 375 380

Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu  
385 390 395 400

Lys Asp Ala Gln Thr Gly Gly Gly Thr Gly Gly Phe Glu Met Ala Ser  
405 410 415

Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr Gly Pro Gln  
420 425 430

Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser Phe Ile Asn  
435 440 445

Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile Phe Leu His  
450 455 460

Gly Asn Ala Thr Ser Ser Tyr Leu Trp Arg His Val Val Pro His Ile  
465 470 475 480

Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly Met Gly Lys  
485 490 495

Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp His Tyr Lys  
500 505 510

Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys Lys Ile Ile  
515 520 525

Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His Tyr Ala Tyr  
530 535 540

Glu His Gln Asp Arg Ile Lys Ala Ile Val His Met Glu Ser Val Val  
545 550 555 560

Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu Glu Asp Ile  
565 570 575

Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu Glu Asn Asn  
580 585 590

Phe Phe Val Glu Thr Val Leu Pro Ser Lys Ile Met Arg Lys Leu Glu  
595 600 605

Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu Lys Gly Glu  
610 615 620

Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro Leu Val Lys  
625 630 635 640

Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr Asn Ala Tyr  
645 650 655

Leu Arg Ala Ser Asp Asp Leu Pro Lys Leu Phe Ile Glu Ser Asp Pro  
660 665 670

Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys Phe Pro Asn  
675 680 685

Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Leu Gln Glu Asp Ala  
690 695 700

Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu Arg Val Leu  
705 710 715 720

Lys Asn Glu Gln

<210> 35  
<211> 1050  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> nucleotide sequence encoding RLuc8Cys4

<400> 35  
atgcgggggtt ctcatcatca tcatcatcat tgcattggcta gcatgactgg tggacagcaa 60  
atgggtcggg atctgtacga cgatgacgat aaggatcgat ggggatccga attcatggct 120  
tccaaggtgt acgacccga gcaacgcaaa cgcatgatca ctgggcctca gtgggtgggct 180  
cgctgcaagc aaatgaacgt gctggactcc ttcattcaact actatgattc cgagaagcac 240  
gccgagaacg ccgtgatttt tctgcatggg aacgctacct ccagctacct gtggaggcac 300  
gtcgtgcctc acatcgagcc cgtgggctaga tgcattcatcc ctgatctgat cggaatgggt 360  
aagtccggca agagcgggaa tgggtcatat cgcctcctgg atcactacaa gtacctcacc 420  
gcttggttcg agctgctgaa ccttccaaag aaaatcatct ttgtgggcca cgactggggg 480  
gctgctctgg cctttcacta cgcctacgag caccaagaca ggatcaaggc catcgtccat 540  
atggagagtg tcgtggacgt gatcgagtcc tgggacgagt ggcctgacat cgaggaggat 600  
atgccctga tcaagagcga agagggcgag aaaatggtgc ttgagaataa cttcttcgtc 660  
gagaccgtgc tccaagcaa gatcatgcgg aaactggagc ctgaggagtt cgctgcctac 720  
ctggagccat tcaaggagaa gggcgagggt agacggccta ccctctcctg gcctcgcgag 780  
atccctctcg ttaagggagg caagcccgac gtcgtccaga ttgtccgaa ctacaacgcc 840  
taccttcggg ccagcgacga tctgcctaag ctgttcacat agtccgacct tgggttcttt 900  
tccaacgcta ttgtcgaggg agctaagaag ttccctaaca ccgagttcgt gaaggtgaag 960  
ggcctccact tcctccagga ggacgctcca gatgaaatgg gtaagtacat caagagcttc 1020  
gtggagcgcg tgctgaagaa cgagcagtaa 1050

<210> 36  
<211> 1050

<212> DNA  
<213> Artificial Sequence

<220>  
<223> nucleotide sequence encoding RLuc8Cys5

<400> 36  
atgtgcggtt ctcatcatca tcatcatcat ggtatggcta gcatgactgg tggacagcaa 60  
atgggtcggg atctgtacga cgatgacgat aaggatcgat ggggatccga attcatggct 120  
tccaaggtgt acgaccccga gcaacgcaaa cgcatgatca ctgggcctca gtgggtgggct 180  
cgctgcaagc aaatgaacgt gctggactcc ttcatcaact actatgattc cgagaagcac 240  
gccgagaacg ccgtgatttt tctgcatggg aacgctacct ccagctacct gtggaggcac 300  
gtcgtgcctc acatcgagcc cgtggctaga tgcacatcc ctgatctgat cggaatgggt 360  
aagtccggca agagcgggaa tggctcatat cgcctcctgg atcactacaa gtacctcacc 420  
gcttggttcg agctgctgaa ctttccaaag aaaatcatct ttgtgggcca cgactggggg 480  
gctgctctgg cttttcacta cgcctacgag caccaagaca ggatcaaggc catcgtccat 540  
atggagagtg tcgtggacgt gatcgagtcc tgggacgagt ggcctgacat cgaggaggat 600  
atgccctga tcaagagcga agagggcgag aaaatggtgc ttgagaataa cttcttcgtc 660  
gagaccgtgc tccaagcaa gatcatgcgg aaactggagc ctgaggagtt cgctgcctac 720  
ctggagccat tcaaggagaa gggcgagggt agacggccta ccctctcctg gcctcgcgag 780  
atccctctcg ttaagggagg caagcccgac gtcgtccaga ttgtccgcaa ctacaacgcc 840  
taccttcggg ccagcgacga tctgcctaag ctgttcacat agtccgaccc tgggttcttt 900  
tccaacgcta ttgtcgaggg agctaagaag ttccctaaca ccgagttcgt gaaggtgaag 960  
ggcctccact tcctccagga ggacgtcca gatgaaatgg gtaagtacat caagagcttc 1020  
gtggagcgcg tgctgaagaa cgagcagtaa 1050

<210> 37  
<211> 2175  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> nucleotide sequence encoding MBP(K239C)RLuc8

<400> 37  
atgcgggggtt ctcatcatca tcatcatcat ggtatggcta gcatgactgg tggacagcaa 60

|                                                                    |      |
|--------------------------------------------------------------------|------|
| atgggtcggg atctgtacga cgatgacgat aaggatcgat ggggatccga attcatgaaa  | 120  |
| atcgaagaag gtaaactggg aatctggatt aacggcgata aaggctataa cggctctcgct | 180  |
| gaagtcggta agaaattcga gaaagatacc ggaattaaag tcaccgttga gcatccggat  | 240  |
| aaactggaag agaaattccc acaggttgcg gcaactggcg atggccctga cattatcttc  | 300  |
| tgggcacacg accgctttgg tggctacgct caatctggcc tgttggctga aatcaccccg  | 360  |
| gacaaagcgt tccaggacaa gctgtatccg ttacctggg atgccgtacg ttacaacggc   | 420  |
| aagctgattg cttacccgat cgctgttgaa gcgttatcgc tgatttataa caaagatctg  | 480  |
| ctgccgaacc cgccaaaaac ctgggaagag atcccggcgc tggataaaga actgaaagcg  | 540  |
| aaaggtaaga gcgcgctgat gttcaacctg caagaaccgt acttcacctg gccgctgatt  | 600  |
| gctgctgacg ggggttatgc gttcaagtat gaaaacggca agtacgacat taaagacgtg  | 660  |
| ggcgtggata acgctggcgc gaaagcgggt ctgaccttcc tggttgacct gattaanaac  | 720  |
| aaacacatga atgcagacac cgattactcc atcgcagaag ctgcctttaa taaaggcgaa  | 780  |
| acagcgatga ccatcaacgg cccgtgggca tggccaaca tcgacaccag ctgcgtgaat   | 840  |
| tatggtgtaa cggctactgcc gaccttcaag ggtcaaccat ccaaaccgtt cgttggcgtg | 900  |
| ctgagcgag gtattaacgc cgccagtccg aacaaagagc tggcaaaaga gttcctcgaa   | 960  |
| aactatctgc tgactgatga aggtctggaa gcggttaata aagacaaacc gctgggtgcc  | 1020 |
| gtagcgctga agtcttacga ggaagagttg gtgaaagatc cgcgtattgc cgccactatg  | 1080 |
| gaaaacgccc agaaaggatga aatcatgccg aacatcccgc agatgtccgc tttctggtat | 1140 |
| gccgtgcgta ctgcggtgat caacgccgcc agcggtcgtc agactgtcga tgaagccctg  | 1200 |
| aaagacgcgc agactggcgg cgggtaccgtt ggattcgaaa tggcttccaa ggtgtacgac | 1260 |
| cccgagcaac gcaaacgcat gatcactggg cctcagtggg gggctcgtg caagcaaatg   | 1320 |
| aacgtgctgg actccttcat caactactat gattccgaga agcacgccga gaacgccgtg  | 1380 |
| atctttctgc atggtaacgc tacctccagc tacctgtgga ggcacgtcgt gcctcacatc  | 1440 |
| gagcccgtgg ctagatgcat catccctgat ctgatcgga tgggtaagtc cggcaagagc   | 1500 |
| gggaatggct catatgcct cctggatcac tacaagtacc tcaccgcttg gttcgagctg   | 1560 |
| ctgaaccttc caaagaaaat catctttgtg ggccacgact ggggggctgc tctggccttt  | 1620 |
| cactacgcct acgagcacca agacaggatc aaggccatcg tccatatgga gagtgtcgtg  | 1680 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| gacgtgatcg agtcctggga cgagtggcct gacatcgagg aggatatcgc cctgatcaag | 1740 |
| agcgaagagg gcgagaaaat ggtgcttgag aataacttct tcgtcgagac cgtgctccca | 1800 |
| agcaagatca tgcggaaact ggagcctgag gagttcgctg cctacctgga gccattcaag | 1860 |
| gagaagggcg aggttagacg gcctaccctc tcctggcctc gcgagatccc tctcgttaag | 1920 |
| ggaggcaagc ccgacgtcgt ccagattgtc cgcaactaca acgcctacct tcgggccagc | 1980 |
| gacgatctgc ctaagctgtt catcgagtcc gaccctgggt tcttttccaa cgctattgtc | 2040 |
| gaggagctta agaagttccc taacaccgag ttcgtgaagg tgaagggcct ccacttcctc | 2100 |
| caggaggacg ctccagatga aatgggtaag tacatcaaga gcttcgtgga gcgcgtgctg | 2160 |
| aagaacgagc agtaa                                                  | 2175 |

<210> 38  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RLuc8Cys4-F

|                                      |    |
|--------------------------------------|----|
| <400> 38                             |    |
| tcatcatcat catcattgca tggctagcat gac | 33 |

<210> 39  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RLuc8Cys4-R

|                                      |    |
|--------------------------------------|----|
| <400> 39                             |    |
| gtcatgctag ccatgcaatg atgatgatga tga | 33 |

<210> 40  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> RLuc8Cys5-F

|                                        |    |
|----------------------------------------|----|
| <400> 40                               |    |
| aaggagatat acatatgtgc ggttctcatc atcat | 35 |

<210> 41  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> RLuc8Cys5-R  
  
 <400> 41  
 atgatgatga gaaccgcaca tatgtatatc tcctt 35

<210> 42  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> MBP(K239C)-F  
  
 <400> 42  
 tggccaaca tcgactgcag caaagtgaat tatgg 35

<210> 43  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> MBP(K239C)-R  
  
 <400> 43  
 aacatcgaca ccagctgcgt gaattatggt gtaac 35

<210> 44  
 <211> 38  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <223> linking element containing a cysteine residue  
  
 <400> 44

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
 20 25 30

Arg Trp Gly Ser Glu Cys  
35

<210> 45  
<211> 38  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Linking element containing a cysteine residue

<400> 45

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Cys Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe  
35

<210> 46  
<211> 38  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Linking element containing a cysteine residue

<400> 46

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Cys Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe  
35

<210> 47  
<211> 38  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Linking element containing a cysteine residue

<400> 47

Met Arg Gly Ser His His His His His His Cys Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe  
35

<210> 48

<211> 38

<212> PRT

<213> Artificial Sequence

<220>

<223> Linking element containing a cysteine residue

<400> 48

Met Cys Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Glu Phe  
35

<210> 49

<211> 311

<212> PRT

<213> Artificial Sequence

<220>

<223> RLuc

<400> 49

Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
1 5 10 15

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
35 40 45

Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val  
50 55 60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly  
65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His  
115 120 125

Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu  
130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
145 150 155 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
165 170 175

Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg  
180 185 190

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
195 200 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
210 215 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
225 230 235 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu  
245 250 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
260 265 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln  
275 280 285

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
290 295 300

Arg Val Leu Lys Asn Glu Gln  
305 310

<210> 50  
<211> 311  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> RLuc8

<400> 50

Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
1 5 10 15

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
35 40 45

Phe Leu His Gly Asn Ala Thr Ser Ser Tyr Leu Trp Arg His Val Val  
50 55 60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly  
65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His  
115 120 125

Tyr Ala Tyr Glu His Gln Asp Arg Ile Lys Ala Ile Val His Met Glu  
130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
145 150 155 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
165 170 175

Glu Asn Asn Phe Phe Val Glu Thr Val Leu Pro Ser Lys Ile Met Arg  
180 185 190

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
195 200 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
210 215 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
225 230 235 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Leu Phe Ile Glu  
245 250 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
260 265 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Leu Gln  
275 280 285

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
290 295 300

Arg Val Leu Lys Asn Glu Gln  
305 310

<210> 51  
<211> 311  
<212> PRT  
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<220>  
<223> RLuc2

<400> 51

Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
1 5 10 15

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
35 40 45

Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val  
50 55 60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly  
65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His  
115 120 125

Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu  
130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
145 150 155 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
165 170 175

Glu Asn Asn Phe Phe Val Glu Thr Val Leu Pro Ser Lys Ile Met Arg  
180 185 190

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
195 200 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
210 215 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Ala Ile Val Arg Asn Tyr  
225 230 235 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu  
245 250 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
260 265 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln  
275 280 285

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
290 295 300

Arg Val Leu Lys Asn Glu Gln  
305 310