

91200-889617-005610PC\_SequenceListing.TXT  
SEQUENCE LISTING

<110> Thanos, Christopher D.  
Murray, Christopher J.  
Yang, Junhao  
Stephenson, Heather  
Sutro Biopharma, Inc.

<120> Proteolytic Inactivation of Select Proteins  
in Bacterial Extracts for Improved Expression

<130> 91200-889617

<140> WO Not yet assigned

<141> Not yet assigned

<150> US 61/713,245

<151> 2012-10-12

<160> 172

<170> FastSEQ for windows Version 4.0

<210> 1

<211> 360

<212> PRT

<213> Escherichia coli

<220>

<223> Release Factor 1 (RF1)

<400> 1

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Glu Arg Phe Arg Ala Leu Ser Arg Glu Tyr Ala Gln Leu Ser Asp Val
 35      40      45
Ser Arg Cys Phe Thr Asp Trp Gln Gln Val Gln Glu Asp Ile Glu Thr
 50      55      60
Ala Gln Met Met Leu Asp Asp Pro Glu Met Arg Glu Met Ala Gln Asp
 65      70      75      80
Glu Leu Arg Glu Ala Lys Glu Lys Ser Glu Gln Leu Glu Gln Gln Leu
 85      90      95
Gln Val Leu Leu Leu Pro Lys Asp Pro Asp Asp Glu Arg Asn Ala Phe
 100      105      110
Leu Glu Val Arg Ala Gly Thr Gly Asp Glu Ala Ala Leu Phe Ala
 115      120      125
Gly Asp Leu Phe Arg Met Tyr Ser Arg Tyr Ala Glu Ala Arg Arg Trp
 130      135      140
Arg Val Glu Ile Met Ser Ala Ser Glu Gly Glu His Gly Gly Tyr Lys
 145      150      155      160
Glu Ile Ile Ala Lys Ile Ser Gly Asp Gly Val Tyr Gly Arg Leu Lys
 165      170      175
Phe Glu Ser Gly Gly His Arg Val Gln Arg Val Pro Ala Thr Glu Ser
 180      185      190
Gln Gly Arg Ile His Thr Ser Ala Cys Thr Val Ala Val Met Pro Glu
 195      200      205
Leu Pro Asp Ala Glu Leu Pro Asp Ile Asn Pro Ala Asp Leu Arg Ile
 210      215      220
Asp Thr Phe Arg Ser Ser Gly Ala Gly Gly Gln His Val Asn Thr Thr
 225      230      235      240
Asp Ser Ala Ile Arg Ile Thr His Leu Pro Thr Gly Ile Val Val Glu
 245      250      255
Cys Gln Asp Glu Arg Ser Gln His Lys Asn Lys Ala Lys Ala Leu Ser
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| Ala | Glu | 275 | Ala | Ser | Thr | Arg | Arg | 280 | Asn | Leu | Leu | Gly | Ser | 285 | Gly | Asp | Arg | Ser |
|     | 290 |     |     |     |     |     | 295 |     |     |     |     |     | 300 |     |     |     |     |     |
| Asp | Arg | Asn | Arg | Thr | Tyr | Asn | Phe | Pro | Gln | Gly | Arg | Val | Thr | Asp | His |     |     |     |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |     |     |     |
| Arg | Ile | Asn | Leu | Thr | Leu | Tyr | Arg | Leu | Asp | Glu | Val | Met | Glu | Gly | Lys |     |     |     |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |     |     |     |
| Leu | Asp | Met | Leu | Ile | Glu | Pro | Ile | Ile | Gln | Glu | His | Gln | Ala | Asp | Gln |     |     |     |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |     |     |     |
| Leu | Ala | Ala | Leu | Ser | Glu | Gln | Glu |     |     |     |     |     |     |     |     |     |     |     |
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 <213> Escherichia coli

<220>  
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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
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| Met | Phe | Glu | Ile | Asn | Pro | Val | Asn | Asn | Arg | Ile | Gln | Asp | Leu | Thr | Glu |  |  |  |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |  |  |  |
| Arg | Ser | Asp | Val | Leu | Arg | Gly | Tyr | Leu | Asp | Tyr | Asp | Ala | Lys | Lys | Glu |  |  |  |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |  |  |  |
| Arg | Leu | Glu | Glu | Val | Asn | Ala | Glu | Leu | Glu | Gln | Pro | Asp | Val | Trp | Asn |  |  |  |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |  |  |
| Glu | Pro | Glu | Arg | Ala | Gln | Ala | Leu | Gly | Lys | Glu | Arg | Ser | Ser | Leu | Glu |  |  |  |
|     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |  |  |  |
| Ala | Val | Val | Asp | Thr | Leu | Asp | Gln | Met | Lys | Gln | Gly | Leu | Glu | Asp | Val |  |  |  |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |  |  |
| Ser | Gly | Leu | Leu | Glu | Leu | Ala | Val | Glu | Ala | Asp | Asp | Glu | Glu | Thr | Phe |  |  |  |
|     |     |     |     | 85  |     |     |     | 90  |     |     |     |     |     | 95  |     |  |  |  |
| Asn | Glu | Ala | Val | Ala | Glu | Leu | Asp | Ala | Leu | Glu | Glu | Lys | Leu | Ala | Gln |  |  |  |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |  |  |
| Leu | Glu | Phe | Arg | Arg | Met | Phe | Ser | Gly | Glu | Tyr | Asp | Ser | Ala | Asp | Cys |  |  |  |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |  |  |
| Tyr | Leu | Asp | Ile | Gln | Ala | Gly | Ser | Gly | Gly | Thr | Glu | Ala | Gln | Asp | Trp |  |  |  |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |  |  |
| Ala | Ser | Met | Leu | Glu | Arg | Met | Tyr | Leu | Arg | Trp | Ala | Glu | Ser | Arg | Gly |  |  |  |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |  |  |
| Phe | Lys | Thr | Glu | Ile | Glu | Glu | Ser | Glu | Gly | Glu | Val | Ala | Gly | Ile |     |  |  |  |
|     |     |     |     | 165 |     |     |     | 170 |     |     |     |     |     | 175 |     |  |  |  |
| Lys | Ser | Val | Thr | Ile | Lys | Ile | Ser | Gly | Asp | Tyr | Ala | Tyr | Gly | Trp | Leu |  |  |  |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |  |  |
| Arg | Thr | Glu | Thr | Gly | Val | His | Arg | Leu | Val | Arg | Lys | Ser | Pro | Phe | Asp |  |  |  |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |  |
| Ser | Gly | Gly | Arg | Arg | His | Thr | Ser | Phe | Ser | Ser | Ala | Phe | Val | Tyr | Pro |  |  |  |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |  |  |
| Glu | Val | Asp | Asp | Asp | Ile | Asp | Ile | Glu | Ile | Asn | Pro | Ala | Asp | Leu | Arg |  |  |  |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |  |  |
| Ile | Asp | Val | Tyr | Arg | Thr | Ser | Gly | Ala | Gly | Gly | Gln | His | Val | Asn | Arg |  |  |  |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |  |  |
| Thr | Glu | Ser | Ala | Val | Arg | Ile | Thr | His | Ile | Pro | Thr | Gly | Ile | Val | Thr |  |  |  |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |  |
| Gln | Cys | Gln | Asn | Asp | Arg | Ser | Gln | His | Lys | Asn | Lys | Asp | Gln | Ala | Met |  |  |  |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |  |
| Lys | Gln | Met | Lys | Ala | Lys | Leu | Tyr | Glu | Leu | Glu | Met | Gln | Lys | Lys | Asn |  |  |  |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |  |
| Ala | Glu | Lys | Gln | Ala | Met | Glu | Asp | Asn | Lys | Ser | Asp | Ile | Gly | Trp | Gly |  |  |  |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |  |  |
| Ser | Gln | Ile | Arg | Ser | Tyr | Val | Leu | Asp | Asp | Ser | Arg | Ile | Lys | Asp | Leu |  |  |  |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |  |  |  |
| Arg | Thr | Gly | Val | Glu | Thr | Arg | Asn | Thr | Gln | Ala | Val | Leu | Asp | Gly | Ser |  |  |  |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |  |
| Leu | Asp | Gln | Phe | Ile | Glu | Ala | Ser | Leu | Lys | Ala | Gly | Leu |     |     |     |  |  |  |
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 Asn Ala Asp Ile Ser Leu Gly Thr Leu Ser Gly Lys Thr Lys Glu Arg  
 15 20 25  
 Val Tyr Leu Ala Glu Glu Gly Gly Arg Lys Val Ser Gln Leu Asp Trp  
 30 35 40  
 Lys Phe Asn Asn Ala Ala Ile Ile Lys Gly Ala Ile Asn Trp Asp Leu  
 45 50 55 60  
 Met Pro Gln Ile Ser Ile Gly Ala Ala Gly Trp Thr Thr Leu Gly Ser  
 65 70 75  
 Arg Gly Gly Asn Met Val Asp Gln Asp Trp Met Asp Ser Ser Asn Pro  
 80 85 90  
 Gly Thr Trp Thr Asp Glu Ser Arg His Pro Asp Thr Gln Leu Asn Tyr  
 95 100 105  
 Ala Asn Glu Phe Asp Leu Asn Ile Lys Gly Trp Leu Leu Asn Glu Pro  
 110 115 120  
 Asn Tyr Arg Leu Gly Leu Met Ala Gly Tyr Gln Glu Ser Arg Tyr Ser  
 125 130 135 140  
 Phe Thr Ala Arg Gly Gly Ser Tyr Ile Tyr Ser Ser Glu Glu Gly Phe  
 145 150 155  
 Arg Asp Asp Ile Gly Ser Phe Pro Asn Gly Glu Arg Ala Ile Gly Tyr  
 160 165 170  
 Lys Gln Arg Phe Lys Met Pro Tyr Ile Gly Leu Thr Gly Ser Tyr Arg  
 175 180 185  
 Tyr Glu Asp Phe Glu Leu Gly Gly Thr Phe Lys Tyr Ser Gly Trp Val  
 190 195 200  
 Glu Ser Ser Asp Asn Asp Glu His Tyr Asp Pro Gly Lys Arg Ile Thr  
 205 210 215 220  
 Tyr Arg Ser Lys Val Lys Asp Gln Asn Tyr Tyr Ser Val Ala Val Asn  
 225 230 235  
 Ala Gly Tyr Tyr Val Thr Pro Asn Ala Lys Val Tyr Val Glu Gly Ala  
 240 245 250  
 Trp Asn Arg Val Thr Asn Lys Lys Gly Asn Thr Ser Leu Tyr Asp His  
 255 260 265  
 Asn Asn Asn Thr Ser Asp Tyr Ser Lys Asn Gly Ala Gly Ile Glu Asn  
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 Tyr Asn Phe Ile Thr Thr Ala Gly Leu Lys Tyr Thr Phe  
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<210> 5  
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<210> 6  
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 Arg Ser

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<210> 13

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<210> 19

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in the switch loop region of Release Factor 1  
(RF1), amino acids 287 to 304

<400> 44

Gln Gln Trp Gly Gly Arg Trp Ala Arg Lys Lys Gly Thr Ile Gly Asp  
1 5 10 15  
Arg Ser

<210> 45

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic OmpT cleavage peptide sequence inserted  
in the switch loop region of Release Factor 1  
(RF1), amino acids 287 to 304

<400> 45

Gln Gln Ala Trp Gly Gly Arg Trp Ala Arg Lys Lys Gly Thr Ile Asp  
1 5 10 15  
Arg Ser

<210> 46

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic OmpT cleavage peptide sequence inserted  
in the switch loop region of Release Factor 1  
(RF1), amino acids 287 to 304

<400> 46

Gln Gln Ala Glu Trp Gly Gly Arg Trp Ala Arg Lys Lys Gly Thr Ile  
1 5 10 15  
Arg Ser

<210> 47

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic OmpT cleavage peptide

<400> 47

Ala Arg Arg Gly  
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<210> 48

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic OmpT cleavage peptide

<400> 48

Trp Leu Ala Ala Arg Arg Gly Arg Gly  
1 5

<210> 49  
 <211> 12  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic OmpT cleavage peptide

<400> 49  
 Trp Gly Gly Arg Trp Ala Arg Lys Lys Gly Thr Ile  
 1 5 10

<210> 50  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> synthetic C-terminal sequence of short form of  
 lambda phage Gam protein (GamS)

<400> 50  
 Gly Gly Ser His His His His His His  
 1 5

<210> 51  
 <211> 42  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic short form of lambda phage Gam protein  
 (GamS) gene amplification primer

<400> 51  
 atatatcata tgaacgctta ttacattcag gatcgtcttg ag 42

<210> 52  
 <211> 72  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic short form of lambda phage Gam protein  
 (GamS) gene amplification primer

<400> 52  
 atatatgtcg acttaatgat gatgatgatg atgagaaccc cctacctctg aatcaatatc 60  
 aacctggtg tg 72

<210> 53  
 <211> 58  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic 5'-fragment including T7 promoter, constant region  
 of the N-terminal sequence and the mutation site first step  
 PCR amplification oligo primer 5chiT2PT7

<400> 53  
 gcgtactagc gtaccacgtg gctggtggcc gattcattaa tgcagctggc acgacagg 58

<210> 54

<211> 60  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic 3'-fragment including mutation site, constant  
 C-terminal region and T7 terminator sequences first step PCR  
 amplification oligo primer 3chiT2TT7

<400> 54  
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<210> 55  
 <211> 28  
 <212> DNA  
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<220>  
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 overlapping second step PCR single primer 5chiT2

<400> 55  
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<210> 56  
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 <212> DNA  
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<220>  
 <223> synthetic WT RF1 variant OmpT cleavage site  
 screening forward primer

<400> 56  
 gctcgatgat cctgaaatgc gtgagatggc gcagg 35

<210> 57  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic M74R RF1 variant OmpT cleavage site  
 screening forward primer

<400> 57  
 gctcgatgat cctgaacgcc gtgagatggc gcagg 35

<210> 58  
 <211> 38  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> synthetic E76K RF1 variant OmpT cleavage site  
 screening forward primer

<400> 58  
 cgatgatcct gaaatgcgta agatggcgca ggatgaac 38

<210> 59  
 <211> 40  
 <212> DNA  
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<220>  
 <223> synthetic E84K RF1 variant OmpT cleavage site

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screening forward primer

<400> 59  
caggatgaac tgcgcaaagc taaagaaaa agcgagcaac 40

<210> 60  
<211> 40  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic A85R RF1 variant OmpT cleavage site  
screening forward primer

<400> 60  
caggatgaac tgcgcgaacg taaagaaaa agcgagcaac 40

<210> 61  
<211> 44  
<212> DNA  
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<220>  
<223> synthetic E87R RF1 variant OmpT cleavage site  
screening forward primer

<400> 61  
ggatgaactg cgcgaagcta aacgtaaaag cgagcaactg gaac 44

<210> 62  
<211> 38  
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<220>  
<223> synthetic E108R RF1 variant OmpT cleavage site  
screening forward primer

<400> 62  
gccaaaagat cctgatgacc gtcgtaacgc cttcctcg 38

<210> 63  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic T293R RF1 variant OmpT cleavage site  
screening forward primer

<400> 63  
caacaggccg aagcgtctcg ccgtcgtaac ctgc 34

<210> 64  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic N296K RF1 variant OmpT cleavage site  
screening forward primer

<400> 64  
gcgctaccc gtcgtaaact gctggggagt ggcg 34

<210> 65  
<211> 36  
<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic S304K RF1 variant OmpT cleavage site screening forward primer

<400> 65  
gggagtggcg atcgcaagga ccgtaaccgt acttac 36

<210> 66  
<211> 46  
<212> DNA  
<213> Artificial Sequence

<220>

<223> synthetic N296K/L297V RF1 variant OmpT cleavage site screening forward primer

<400> 66  
cgaagcgtct acccgtcgta aagttctggg gagtggcgat cgcagc 46

<210> 67  
<211> 46  
<212> DNA  
<213> Artificial Sequence

<220>

<223> synthetic N296K/L297K RF1 variant OmpT cleavage site screening forward primer

<400> 67  
cgaagcgtct acccgtcgta aaaagctggg gagtggcgat cgcagc 46

<210> 68  
<211> 46  
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<213> Artificial Sequence

<220>

<223> synthetic N296K/L297R RF1 variant OmpT cleavage site screening forward primer

<400> 68  
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<210> 69  
<211> 46  
<212> DNA  
<213> Artificial Sequence

<220>

<223> synthetic N296R/L297V RF1 variant OmpT cleavage site screening forward primer

<400> 69  
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<210> 70  
<211> 46  
<212> DNA  
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<220>

<223> synthetic N296R/L297K RF1 variant OmpT cleavage site screening forward primer

<400> 70  
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<210> 71  
 <211> 46  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> synthetic N296R/L297R RF1 variant OmpT cleavage  
 site screening forward primer  
  
 <400> 71  
 cgaagcgtct acccgctcgtc gccgtctggg gagtggcgat cgcagc 46  
  
 <210> 72  
 <211> 55  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> synthetic N296K/L297R/L298K RF1 variant OmpT  
 cleavage site screening forward primer  
  
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 caggccgaag cgtctacccg tcgtaaacgt aaggggagtg gcgatcgag cgacc 55  
  
 <210> 73  
 <211> 55  
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 <220>  
 <223> synthetic N296K/L297R/L298R RF1 variant OmpT  
 cleavage site screening forward primer  
  
 <400> 73  
 caggccgaag cgtctacccg tcgtaaacgt cgcgggagtg gcgatcgag cgacc 55  
  
 <210> 74  
 <211> 35  
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 <220>  
 <223> synthetic WT RF1 variant OmpT cleavage site  
 screening reverse primer  
  
 <400> 74  
 cctgcgccat ctcacgcatt tcaggatcat cgagc 35  
  
 <210> 75  
 <211> 35  
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 <220>  
 <223> synthetic M74R RF1 variant OmpT cleavage site  
 screening reverse primer  
  
 <400> 75  
 cctgcgccat ctcacggcgt tcaggatcat cgagc 35  
  
 <210> 76  
 <211> 38  
 <212> DNA  
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 <220>  
 <223> synthetic E76K RF1 variant OmpT cleavage site

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screening reverse primer

<400> 76  
gttcatcctg cgccatctta cgcatttcag gatcatcg 38

<210> 77  
<211> 40  
<212> DNA  
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<220>  
<223> synthetic E84K RF1 variant OmpT cleavage site  
screening reverse primer

<400> 77  
gttgctcgct tttttcttta gctttgcgca gttcatcctg 40

<210> 78  
<211> 40  
<212> DNA  
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<220>  
<223> synthetic A85R RF1 variant OmpT cleavage site  
screening reverse primer

<400> 78  
gttgctcgct tttttcttta cgttcgcgca gttcatcctg 40

<210> 79  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic E87R RF1 variant OmpT cleavage site  
screening reverse primer

<400> 79  
gttccagttg ctcgctttta cgtttagctt cgcgagttc atcc 44

<210> 80  
<211> 38  
<212> DNA  
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<220>  
<223> synthetic E108R RF1 variant OmpT cleavage site  
screening reverse primer

<400> 80  
cgaggaaggc gttacgacgg tcatcaggat cttttggc 38

<210> 81  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic T293R RF1 variant OmpT cleavage site  
screening reverse primer

<400> 81  
gcaggttacg acggcgagac gcttcggcct gttg 34

<210> 82  
<211> 34  
<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic N296K RF1 variant OmpT cleavage site  
screening reverse primer

<400> 82  
cgccactccc cagcagttta cgacgggtag acgc 34

<210> 83  
<211> 36  
<212> DNA  
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<220>

<223> synthetic S304K RF1 variant OmpT cleavage site  
screening reverse primer

<400> 83  
gtaagtacgg ttacggtcct tgcgatcgcc actccc 36

<210> 84  
<211> 46  
<212> DNA  
<213> Artificial Sequence

<220>

<223> synthetic N296K/L297V RF1 variant OmpT cleavage  
site screening reverse primer

<400> 84  
gctgcgatcg ccactcccca gaactttacg acgggtagac gcttcg 46

<210> 85  
<211> 46  
<212> DNA  
<213> Artificial Sequence

<220>

<223> synthetic N296K/L297K RF1 variant OmpT cleavage  
site screening reverse primer

<400> 85  
gctgcgatcg ccactcccca gctttttacg acgggtagac gcttcg 46

<210> 86  
<211> 46  
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<220>

<223> synthetic N296K/L297R RF1 variant OmpT cleavage  
site screening reverse primer

<400> 86  
gctgcgatcg ccactcccca gacgtttacg acgggtagac gcttcg 46

<210> 87  
<211> 46  
<212> DNA  
<213> Artificial Sequence

<220>

<223> synthetic N296R/L297V RF1 variant OmpT cleavage  
site screening reverse primer

<400> 87  
gctgcgatcg ccactcccca gaacgcgacg acgggtagac gcttcg 46

<210> 88  
 <211> 46  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> synthetic N296R/L297K RF1 variant OmpT cleavage  
 site screening reverse primer  
  
 <400> 88  
 gctgcgatcg ccactcccca gcttgcgacg acgggtagac gcttcg 46  
  
 <210> 89  
 <211> 46  
 <212> DNA  
 <213> Artificial Sequence  
  
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 <223> synthetic N296R/L297R RF1 variant OmpT cleavage  
 site screening reverse primer  
  
 <400> 89  
 gctgcgatcg ccactcccca gacggcgacg acgggtagac gcttcg 46  
  
 <210> 90  
 <211> 55  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> synthetic N296K/L297R/L298K RF1 variant OmpT  
 cleavage site screening reverse primer  
  
 <400> 90  
 ggtcgctgcg atcgccactc cccttacgtt tacgacgggt agacgcttcg gcctg 55  
  
 <210> 91  
 <211> 55  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> synthetic N296K/L297R/L298R RF1 variant OmpT  
 cleavage site screening reverse primer  
  
 <400> 91  
 ggtcgctgcg atcgccactc ccgcgacgtt tacgacgggt agacgcttcg gcctg 55  
  
 <210> 92  
 <211> 75  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> synthetic No. 0 RF1 variant OmpT cleavage peptide  
 insertion forward oligo primer  
  
 <400> 92  
 gccgaagcgt ctaccgctcg taacctgctg gggagtggcg atcgacgcga ccgtaaccgt 60  
 acttacaact tcccg 75  
  
 <210> 93  
 <211> 75  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>

<223> synthetic No. 1 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

<400> 93  
cgccgtgggt ctaccgctcg taacctgctg gggagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 94  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic No. 2 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

<400> 94  
gcacgccgtg gtaccgctcg taacctgctg gggagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 95  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic No. 3 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

<400> 95  
gccgcacgcc gtggctgctg taacctgctg gggagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 96  
<211> 75  
<212> DNA  
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<220>  
<223> synthetic No. 4 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

<400> 96  
gccgaagcac gccgtggctg taacctgctg gggagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 97  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic No. 5 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

<400> 97  
gccgaagcgg cagccgtgg taacctgctg gggagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 98  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic No. 6 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

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<400> 98  
gccgaagcgt ctgcacgccg tggctctgctg gggagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 99  
<211> 75  
<212> DNA  
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<220>  
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insertion forward oligo primer

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gccgaagcgt ctaccgcacg ccgtggtctg gggagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 100  
<211> 75  
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<220>  
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insertion forward oligo primer

<400> 100  
gccgaagcgt ctacccgtgc acgccgtggt gggagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 101  
<211> 75  
<212> DNA  
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<220>  
<223> synthetic No. 9 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

<400> 101  
gccgaagcgt ctacccgtcg tgcacgccgt ggtagtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 102  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic No. 10 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

<400> 102  
gccgaagcgt ctacccgtcg taacgcacgc cgtggtggcg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

<210> 103  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic No. 11 RF1 variant OmpT cleavage peptide  
insertion forward oligo primer

<400> 103  
gccgaagcgt ctacccgtcg taacctggca cgccgtggtg atcgcagcga ccgtaaccgt 60  
acttacaact tcccg 75

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<210> 104  
 <211> 75  
 <212> DNA  
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 <223> synthetic No. 12 RF1 variant OmpT cleavage peptide  
 insertion forward oligo primer  
  
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 acttacaact tcccg 75  
  
 <210> 105  
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 insertion forward oligo primer  
  
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 gccgaagcgt ctacccgtcg taacctgctg ggggcacgcc gtggtagcga ccgtaaccgt 60  
 acttacaact tcccg 75  
  
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 <223> synthetic No. 14 RF1 variant OmpT cleavage peptide  
 insertion forward oligo primer  
  
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 acttacaact tcccg 75  
  
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 insertion forward oligo primer  
  
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 acttacaact tcccg 75  
  
 <210> 108  
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 <212> DNA  
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 <220>  
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 insertion forward oligo primer  
  
 <400> 108  
 gccgaagcgt ctacccgtcg tcgtctgctg gggagtggcg atcgacgcga ccgtaaccgt 60  
 acttacaact tcccg 75  
  
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<212> DNA
<213> Artificial Sequence

<220>
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gcctggctgg cagcgcgtcg cggtcgtggc gggagtggcg atcgcagcga ccgtaaccgt 60
acttacaact tcccg                                         75

<210> 110
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 18 RF1 variant OmpT cleavage peptide
      insertion forward oligo primer

<400> 110
gccgaatggc tggcagcgcg tcgcggtcgt ggcagtggcg atcgcagcga ccgtaaccgt 60
acttacaact tcccg                                         75

<210> 111
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 19 RF1 variant OmpT cleavage peptide
      insertion forward oligo primer

<400> 111
gccgaagcgt ggctggcagc gcgtcgcggt cgtggcgggcg atcgcagcga ccgtaaccgt 60
acttacaact tcccg                                         75

<210> 112
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 20 RF1 variant OmpT cleavage peptide
      insertion forward oligo primer

<400> 112
tgggggtggc gttgggctcg caagaaaggt actattggcg atcgcagcga ccgtaaccgt 60
acttacaact tcccg                                         75

<210> 113
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 21 RF1 variant OmpT cleavage peptide
      insertion forward oligo primer

<400> 113
gcctgggggtg gccgttgggc tcgcaagaaa ggtactattg atcgcagcga ccgtaaccgt 60
acttacaact tcccg                                         75

<210> 114
<211> 75
<212> DNA
<213> Artificial Sequence

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<220>
<223> synthetic No. 22 RF1 variant OmpT cleavage peptide
      insertion forward oligo primer

<400> 114
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acttacaact tcccg                                         75

<210> 115
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 0 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 115
gctgcgatcg ccactcccca gcaggttacg acgggtagac gcttcggcct gttggcgttt 60
tgccatttca gcagc                                         75

<210> 116
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 1 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 116
gctgcgatcg ccactcccca gcaggttacg acgggtagaa ccacggcgtg cttggcgttt 60
tgccatttca gcagc                                         75

<210> 117
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 2 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 117
gctgcgatcg ccactcccca gcaggttacg acgggtacca cggcgtgcct gttggcgttt 60
tgccatttca gcagc                                         75

<210> 118
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 3 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 118
gctgcgatcg ccactcccca gcaggttacg acgaccacgg cgtgcggcct gttggcgttt 60
tgccatttca gcagc                                         75

<210> 119
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 4 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

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<400> 119  
gctgcatcg ccactcccca gcaggttacg accacggcgt gcttcggcct gttggcgttt 60  
tgccatttca gcagc 75

<210> 120  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> synthetic No. 5 RF1 variant OmpT cleavage peptide  
insertion reverse oligo primer

<400> 120  
gctgcatcg ccactcccca gcaggttacc acggcgtgcc gcttcggcct gttggcgttt 60  
tgccatttca gcagc 75

<210> 121  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
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insertion reverse oligo primer

<400> 121  
gctgcatcg ccactcccca gcagaccacg gcgtgcagac gcttcggcct gttggcgttt 60  
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<210> 122  
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insertion reverse oligo primer

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insertion reverse oligo primer

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insertion reverse oligo primer

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75

&lt;210&gt; 125

&lt;211&gt; 75

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&lt;220&gt;

<223> synthetic No. 10 RF1 variant OmpT cleavage peptide  
insertion reverse oligo primer

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tgccatttca gcagc 75

&lt;210&gt; 126

&lt;211&gt; 75

&lt;212&gt; DNA

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&lt;220&gt;

<223> synthetic No. 11 RF1 variant OmpT cleavage peptide  
insertion reverse oligo primer

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tgccatttca gcagc 75

&lt;210&gt; 127

&lt;211&gt; 75

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> synthetic No. 12 RF1 variant OmpT cleavage peptide  
insertion reverse oligo primer

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tgccatttca gcagc 75

&lt;210&gt; 128

&lt;211&gt; 75

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

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insertion reverse oligo primer

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tgccatttca gcagc 75

&lt;210&gt; 129

&lt;211&gt; 75

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> synthetic No. 14 RF1 variant OmpT cleavage peptide  
insertion reverse oligo primer

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tgccatttca gcagc 75

&lt;210&gt; 130

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 insertion reverse oligo primer  
  
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 insertion reverse oligo primer  
  
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 insertion reverse oligo primer  
  
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tgccatttca gcagc 75

<210> 136
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tgccatttca gcagc 75

<210> 137
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tgccatttca gcagc 75

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gaagcc 66

<210> 139
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<400> 140

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amber codon in Herceptin

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38

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<400> 172

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38