5' tggcgaatgggacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacgcgcagcgtgacc 70
3' accgcttaccctgcgcgggacatcgccgcgtaattcgcgccgcccacaccaccaatgcgcgtcgcactgg

5' gctacacttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccg 140
3' cgatgtgaacggtcgcgggatcgcgggcgaggaaagcgaaagaagggaaggaaagagcggtgcaagcggc

5' gctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagtgctttacggcacctcga 210
3' cgaaaggggcagttcgagatttagcccccgagggaaatcccaaggctaaatcacgaaatgccgtggagct

DraIII
5' ccccaaaaaacttgattagggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccct 280
3' ggggttttttgaactaatcccactaccaagtgcatcacccggtagcgggactatctgccaaaaagcggga

AloI' AloI
5' ttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacactcaaccctatct 350
3' aactgcaacctcaggtgcaagaaattatcacctgagaacaaggtttgaccttgttgtgagttgggataga

5' cggtctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgattta 420
3' gccagataagaaaactaaatattccctaaaacggctaaagccggataaccaattttttactcgactaaat

5' acaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatg 490
3' tgtttttaaattgcgcttaaaattgttttataattgcgaatgttaaatccaccgtgaaaagcccctttac

5' tgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataacc 560
3' acgcgccttggggataaacaaataaaaagatttatgtaagtttatacataggcgagtactctgttattgg

5' ctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttatt 630
3' gactatttacgaagttattataactttttccttctcatactcataagttgtaaaggcacagcgggaataa
Ap

5' cccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctg 700
3' gggaaaaaacgccgtaaaacggaaggacaaaaacgagtgggtctttgcgaccactttcattttctacgac
Ap

5' aagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttt 770
3' ttctagtcaacccacgtgctcacccaatgtagcttgacctagagttgtcgccattctaggaactctcaaa
Ap

```
5' tcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgt   840
3' agcggggcttcttgcaaaaggttactactcgtgaaaatttcaagacgatacaccgcgccataatagggca
   Ap

                                                                    ScaI
5' attgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcac   910
3' taactgcggcccgttctcgttgagccagcggcgtatgtgataagagtcttactgaaccaactcatgagtg
   Ap

5' cagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgag   980
3' gtcagtgtcttttcgtagaatgcctaccgtactgtcattctcttaatacgtcacgacggtattggtactc
   Ap

                                    PvuI
5' tgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcac   1050
3' actattgtgacgccggttgaatgaagactgttgctagcctcctggcttcctcgattggcgaaaaaacgtg
   Ap

5' aacatgggggatcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccataccaaacgacg   1120
3' ttgtaccccctagtacattgagcggaactagcaacccttggcctcgacttacttcggtatggtttgctgc
   Ap

5' agcgtgacaccacgatgcctgcagcaatggcaacaacgttgcgcaaactattaactggcgaactacttac   1190
3' tcgcactgtggtgctacggacgtcgttaccgttgttgcaacgcgtttgataattgaccgcttgatgaatg
   Ap

5' tctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcg   1260
3' agatcgaagggccgttgttaattatctgacctacctccgcctatttcaacgtcctggtgaagacgcgagc
   Ap

                                                               BsaI
5' gcccttccggctggctggtttattgctgataaatctggagccggtgagcgtgggtctcgcggtatcattg   1330
3' cgggaaggccgaccgaccaaataacgactatttagacctcggccactcgcacccagagcgccatagtaac
   Ap

                                                         AhdI
5' cagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactat   1400
3' gtcgtgaccccggtctaccattcgggagggcatagcatcaatagatgtgctgcccctcagtccgttgata
   Ap
```

5' ggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaa 1470
3' cctacttgctttatctgtctagcgactctatccacggagtgactaattcgtaaccattgacagtctggtt
Ap

5' gtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcc 1540
3' caaatgagtatatatgaaatctaactaaattttgaagtaaaaattaaattttcctagatccacttctagg

5' tttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtaga 1610
3' aaaaactattagagtactggttttagggaattgcactcaaaagcaaggtgactcgcagtctggggcatct

5' aaagatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaacca 1680
3' tttctagtttcctagaagaactctaggaaaaaaagacgcgcattagacgacgaacgtttgtttttttggt
Ori

5' ccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttca 1750
3' ggcgatggtcgccaccaaacaaacggcctagttctcgatggttgagaaaaaggcttccattgaccgaagt
Ori

5' gcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgt 1820
3' cgtctcgcgtctatggtttatgacaggaagatcacatcggcatcaatccggtggtgaagttcttgagaca
Ori

5' agcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgt 1890
3' tcgtggcggatgtatggagcgagacgattaggacaatggtcaccgacgacggtcaccgctattcagcaca
Ori

5' cttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacggggggttcgt 1960
3' gaatggcccaacctgagttctgctatcaatggcctattccgcgtcgccagcccgacttgccccccaagca
Ori

5' gcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaag 2030
3' cgtgtgtcgggtcgaacctcgcttgctggatgtggcttgactctatggatgtcgcactcgatactctttc
Ori

5' cgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgc 2100
3' gcggtgcgaagggcttccctctttccgcctgtccataggccattcgccgtcccagccttgtcctctcgcg
Ori

```
5'  acgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttg
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2170
3'  tgctccctcgaaggtcccccttttgcggaccatagaaatatcaggacagcccaaagcggtggagactgaac
o                                   Ori
o
5'  agcgtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggcctttttt
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2240
3'  tcgcagctaaaaacactacgagcagtcccccgcctcggatacctttttgcggtcgttgcgccggaaaaa
o                                   Ori
o
o                                      PciI
5'  acggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggat
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2310
3'  tgccaaggaccggaaaacgaccggaaaacgagtgtacaagaaaggacgcaatagggactaagacaccta
o
5'  aaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtcag
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2380
3'  ttggcataatggcggaaactcactcgactatggcgagcggcgtcggcttgctggctcgcgtcgctcagtc
o
o                 SapI
5'  tgagcgaggaagcggaagagcgcctgatgcggtattttctccttacgcatctgtgcggtatttcacaccg
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2450
3'  actcgctccttcgccttctcgcggactacgccataaaagaggaatgcgtagacacgccataaagtgtggc
o
o                                                          BstZ17I
5'  catatatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatacactccgctatc
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2520
3'  gtatataccacgtgagagtcatgttagacgagactacggcgtatcaattcggtcatatgtgaggcgatag
o
o                  Tth111I
5'  gctacgtgactgggtcatggctgcgccccgacacccgccaacacccgctgacgcgccctgacgggcttgt
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2590
3'  cgatgcactgacccagtaccgacgcggggctgtgggcggttgtgggcgactgcgcgggactgcccgaaca
o
5'  ctgctcccggcatccgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcac
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2660
3'  gacgagggccgtaggcgaatgtctgttcgacactggcagaggccctcgacgtacacagtctccaaaagtg
o
5'  cgtcatcaccgaaacgcgcgaggcagctgcggtaaagctcatcagcgtggtcgtgaagcgattcacagat
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2730
3'  gcagtagtggctttgcgcgctccgtcgacgccatttcgagtagtcgcaccagcacttcgctaagtgtcta
o
5'  gtctgcctgttcatccgcgtccagctcgttgagtttctccagaagcgttaatgtctggcttctgataaag
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2800
3'  cagacggacaagtaggcgcaggtcgagcaactcaaagaggtcttcgcaattacagaccgaagactatttc
o
5'  cgggccatgttaagggcggtttttcctgtttggtcactgatgcctccgtgtaagggggatttctgttca
o   ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  2870
3'  gcccggtacaattcccgccaaaaaaggacaaaccagtgactacggaggcacattccccctaaagacaagt
o
```

```
5' tggggtaatgataccgatgaaacgagagaggatgctcacgatacgggttactgatgatgaacatgcccg
                                                                          2940
3' acccccattactatggctactttgctctctcctacgagtgctatgcccaatgactactacttgtacgggc

5' gttactggaacgttgtgagggtaaacaactggcggtatggatgcggcgggaccagagaaaaatcactcag
                                                                          3010
3' caatgaccttgcaacactcccatttgttgaccgccatacctacgccgccctggtctctttttagtgagtc

5' ggtcaatgccagcgcttcgttaatacagatgtaggtgttccacagggtagccagcagcatcctgcgatgc
                                                                          3080
3' ccagttacggtcgcgaagcaattatgtctacatccacaggtgtcccatcggtcgtcgtaggacgctacg

5' agatccggaacataatggtgcagggcgctgacttccgcgtttccagactttacgaaacacggaaaccgaa
                                                                          3150
3' tctaggccttgtattaccacgtcccgcgactgaaggcgcaaaggtctgaaatgctttgtgcctttggctt

                    Bpu10I
5' gaccattcatgttgttgctcaggtcgcagacgttttgcagcagcagtcgcttcacgttcgctcgcgtatc
                                                                          3220
3' ctggtaagtacaacaacgagtccagcgtctgcaaaacgtcgtcgtcagcgaagtgcaagcgagcgcatag

5' ggtgattcattctgctaaccagtaaggcaaccccgccagcctagccgggtcctcaacgacaggagcacga
                                                                          3290
3' ccactaagtaagacgattggtcattccgttggggcggtcggatcggcccaggagttgctgtcctcgtgct

      FspAI
5' tcatgcgcacccgtggggccgccatgccggcgataatggcctgcttctcgccgaaacgtttggtggcggg
                                                                          3360
3' agtacgcgtgggcaccccggcggtacggccgctattaccggacgaagagcggctttgcaaaccaccgccc

5' accagtgacgaaggcttgagcgagggcgtgcaagattccgaataccgcaagcgacaggccgatcatcgtc
                                                                          3430
3' tggtcactgcttccgaactcgctcccgcacgttctaaggcttatggcgttcgctgtccggctagtagcag

5' gcgctccagcgaaagcggtcctcgccgaaaatgacccagagcgctgccggcacctgtcctacgagttgca
                                                                          3500
3' cgcgaggtcgctttcgccaggagcggcttttactgggtctcgcgacggccgtggacaggatgctcaacgt

                                 PshAI
5' tgataaagaagacagtcataagtgcggcgacgatagtcatgccccgcgcccaccggaaggagctgactgg
                                                                          3570
3' actatttcttctgtcagtattcacgccgctgctatcagtacggggcgcgggtggccttcctcgactgacc

5' gttgaaggctctcaagggcatcggtcgagatcccggtgcctaatgagtgagctaacttacattaattgcg
                                                                          3640
3' caacttccgagagttcccgtagccagctctagggccacggattactcactcgattgaatgtaattaacgc

5' ttgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcggccaacgcg
                                                                          3710
3' aacgcgagtgacgggcgaaaggtcagccctttggacagcacggtcgacgtaattacttagccggttgcgc
                                 Lac I
```

5' cggggagaggcggtttgcgtattgggcgccagggtggtttttcttttcaccagtgagacgggcaacagct
3780
3' gcccctctccgccaaacgcataacccgcggtcccaccaaaaagaaaagtggtcactctgcccgttgtcga
Lac I

5' gattgcccttcaccgcctggccctgagagagttgcagcaagcggtccacgctggtttgccccagcaggcg
3850
3' ctaacgggaagtggcggaccgggactctctcaacgtcgttcgccaggtgcgaccaaacggggtcgtccgc
Lac I

HpaI
5' aaaatcctgtttgatggtggttaacggcgggatataacatgagctgtcttcggtatcgtcgtatcccact
3920
3' ttttaggacaaactaccaccaattgccgccctatattgtactcgacagaagccatagcagcatagggtga
Lac I

BssHII
5' accgagatgtccgcaccaacgcgcagcccggactcggtaatggcgcgcattgcgcccagcgccatctgat
3990
3' tggctctacaggcgtggttgcgcgtcgggcctgagccattaccgcgcgtaacgcgggtcgcggtagacta
Lac I

5' cgttggcaaccagcatcgcagtgggaacgatgccctcattcagcatttgcatggtttgttgaaaaccgga
4060
3' gcaaccgttggtcgtagcgtcacccttgctacgggagtaagtcgtaaacgtaccaaacaacttttggcct
Lac I

5' catggcactccagtcgccttcccgttccgctatcggctgaatttgattgcgagtgagatatttatgccag
4130
3' gtaccgtgaggtcagcggaagggcaaggcgatagccgacttaaactaacgctcactctataaatacggtc
Lac I

PspOMI
ApaI
5' ccagccagacgcagacgcgccgagacagaacttaatgggcccgctaacagcgcgatttgctggtgaccca
4200
3' ggtcggtctgcgtctgcgcggctctgtcttgaattacccgggcgattgtcgcgctaaacgaccactgggt
Lac I

5' atgcgaccagatgctccacgcccagtcgcgtaccgtcttcatgggagaaaataatactgttgatgggtgt
4270
3' tacgctggtctacgaggtgcgggtcagcgcatggcagaagtaccctcttttattatgacaactacccaca
Lac I

5' ctggtcagagacatcaagaaataacgccggaacattagtgcaggcagcttccacagcaatggcatcctgg
4340
3' gaccagtctctgtagttctttattgcggccttgtaatcacgtccgtcgaaggtgtcgttaccgtaggacc
Lac I

BclI

5' tcatccagcggatagttaatgatcagcccactgacgcgttgcgcgagaagattgtgcaccgccgctttac 4410
3' agtaggtcgcctatcaattactagtcgggtgactgcgcaacgcgctcttctaacacgtggcggcgaaatg

Lac I

5' aggcttcgacgccgcttcgttctaccatcgacaccaccacgctggcacccagttgatcggcgcgagattt 4480
3' tccgaagctgcggcgaagcaagatggtagctgtggtggtgcgaccgtgggtcaactagccgcgctctaaa

Lac I

5' aatcgccgcgacaatttgcgacggcgcgtgcagggccagactggaggtggcaacgccaatcagcaacgac 4550
3' ttagcggcgctgttaaacgctgccgcgcacgtcccggtctgacctccaccgttgcggttagtcgttgctg

Lac I

5' tgtttgcccgccagttgttgtgccacgcggttgggaatgtaattcagctccgccatcgccgcttccactt 4620
3' acaaacgggcggtcaacaacacggtgcgccaacccttacattaagtcgaggcggtagcggcgaaggtgaa

Lac I

5' tttcccgcgttttcgcagaaacgtggctggcctggttcaccacgcgggaaacggtctgataagagacacc 4690
3' aaagggcgcaaaagcgtctttgcaccgaccggaccaagtggtgcgccctttgccagactattctctgtgg

5' ggcatactctgcgacatcgtataacgttactggtttcacattcaccaccctgaattgactctcttccggg 4760
3' ccgtatgagacgctgtagcatattgcaatgaccaaagtgtaagtggtgggacttaactgagagaaggccc

5' cgctatcatgccataccgcgaaaggttttgcgccattcgatggtgtccgggatctcgacgctctccctta 4830
3' gcgatagtacggtatggcgctttccaaaacgcggtaagctaccacaggccctagagctgcgagagggaat

EcoNI

5' tgcgactcctgcattaggaagcagcccagtagtaggttgaggccgttgagcaccgccgccgcaaggaatg 4900
3' acgctgaggacgtaatccttcgtcgggtcatcatccaactccggcaactcgtggcggcggcgttccttac

SphI

5' gtgcatgcaaggagatggcgcccaacagtcccccggccacggggcctgccaccatacccacgccgaaaca 4970
3' cacgtacgttcctctaccgcgggttgtcagggggccggtgccccggacggtggtatgggtgcggctttgt

5' agcgctcatgagcccgaagtggcgagcccgatcttccccatcggtgatgtcggcgatataggcgccagca 5040
3' tcgcgagtactcgggcttcaccgctcgggctagaaggggtagccactacagccgctatatccgcggtcgt

SgrAI

5' accgcacctgtggcgccggtgatgccggccacgatgcgtccggcgtagaggatcgagatcgatctcgatc 5110
3' tggcgtggacaccgcggccactacggccggtgctacgcaggccgcatctcctagctctagctagagctag

XbaI

5' ccgcgaaattaatacgactcactatagggaattgtgagcggataacaattcccctctagaaataatttt 5180
3' ggcgctttaattatgctgagtgatatcccttaacactcgcctattgttaaggggagatctttattaaaa

T7 Promoter — Lac Operator

BarI — BarI'

5' gtttaactttaagaaggagatatacatatgagcgataaaattattcacctgactgacgacagttttgaca 5250
3' caaattgaaattcttcctctatatgtatactcgctattttaataagtggactgactgctgtcaaaactgt

Trx Tag

M S D K I I H L T D D S F D
1 2 3 4 5 6 7 8 9 10 11 12 13 14

RsrII

5' cggatgtactcaaagcggacggggcgatcctcgtcgatttctgggcagagtggtgcggtccgtgcaaaat 5320
3' gcctacatgagtttcgcctgccccgctaggagcagctaaagacccgtctcaccacgccaggcacgtttta

Trx Tag

T D V L K A D G A I L V D F W A E W C G P C K M
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38

5' gatcgccccgattctggatgaaatcgctgacgaatatcagggcaaactgaccgttgcaaaactgaacatc 5390
3' ctagcggggctaagacctactttagcgactgcttatagtcccgtttgactggcaacgttttgacttgtag

Trx Tag

I A P I L D E I A D E Y Q G K L T V A K L N I
39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61

5' gatcaaaaccctggcactgcgccgaaatatggcatccgtggtatcccgactctgctgctgttcaaaaacg 5460
3' ctagttttgggaccgtgacgcggctttataccgtaggcaccatagggctgagacgacgacaagtttttgc

Trx Tag

D Q N P G T A P K Y G I R G I P T L L L F K N
62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84

```
5'  gtgaagtggcggcaaccaaagtgggtgcactgtctaaaggtcagttgaaagagttcctcgacgctaacct
                                                                          5530
3'  cacttcaccgccgttggtttcacccacgtgacagatttccagtcaactttctcaaggagctgcgattgga
```

Trx Tag

```
G  E  V  A  A  T  K  V  G  A  L  S  K  G  Q  L  K  E  F  L  D  A  N  L
85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108
```

MscI

```
5'  ggccggttctggttctggccatatgcaccatcatcatcatcattcttctggtctggtgccacgcggttct
                                                                          5600
3'  ccggccaagaccaagaccggtatacgtggtagtagtagtagtaagaagaccagaccacggtgcgccaaga
```

T...g | His6-tag | Thrombin

```
A  G  S  G  S  G  H  M  H  H  H  H  H  H  S  S  G  L  V  P  R  G  S
109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131
```

BglII

```
5'  ggtatgaaagaaaccgctgctgctaaattcgaacgccagcacatggacagcccagatctgggtaccgacg
                                                                          5670
3'  ccatactttctttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagacccatggctgc
```

En...e

```
G  M  K  E  T  A  A  A  K  F  E  R  Q  H  M  D  S  P  D  L  G  T  D
132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154
```

NcoI

```
5'  acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttca
                                                                          5740
3'  tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt
```

Enterokinase | attB1 | BGH13_B01b2-pDONR221

```
D  D  D  K  A  M  A  I  S  T  S  L  Y  K  K  A  G  S  E  N  L  Y  F  Q
155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178

5'  aggcCAACCGCGAACCGCGTTCGTTCATTTGTTCGAATGGCAGTGGAACGATATTGCTAGCGAGTGCGAA
                                                                          5810
3'  tccgGTTGGCGCTTGGCGCAAGCAAGTAAACAAGCTTACCGTCACCTTGCTATAACGATCGCTCACGCTT
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BGH13_B01b2-pDONR221

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G  Q  P  R  T  A  F  V  H  L  F  E  W  Q  W  N  D  I  A  S  E  C  E
179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201

5'  AATGTACTGGGGCCAAAAGGCTATGCGGCAGTGCAGGTATCCCCACCCCAAAAATCGGTTAGTAGCAGCC
                                                                          5880
3'  TTACATGACCCCGGTTTTCCGATACGCCGTCACGTCCATAGGGGTGGGGTTTTTAGCCAATCATCGTCGG
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BGH13_B01b2-pDONR221

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N  V  L  G  P  K  G  Y  A  A  V  Q  V  S  P  P  Q  K  S  V  S  S  S
202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224
```

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5'  AATGGTGGAGTCGTTACCAACCTGTTAGCTATGCCATAGAGGGCCGTTCTGGCAATCGCAACGAATTCGC
                                                                      5950
3'  TTACCACCTCAGCAATGGTTGGACAATCGATACGGTATCTCCCGGCAAGACCGTTAGCGTTGCTTAAGCG
    ---------------------------------- BGH13_B01b2-pDONR221 ----------------------------------

    Q  W  W  S  R  Y  Q  P  V  S  Y  A  I  E  G  R  S  G  N  R  N  E  F  A
    225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248

                                                   SnaBI
5'  CAGCATGGTAAGCCGGTGTAAAAATGCTGGGGTGGATATATACGTAGATGCCGTAATTAATCATATGGCT
                                                                      6020
3'  GTCGTACCATTCGGCCACATTTTTACGACCCCACCTATATATGCATCTACGGCATTAATTAGTATACCGA
    ---------------------------------- BGH13_B01b2-pDONR221 ----------------------------------

    S  M  V  S  R  C  K  N  A  G  V  D  I  Y  V  D  A  V  I  N  H  M  A
    249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271

                                                                PmlI
5'  GCCGGCGGGCGCTATTACCCAGAAGTGCCTTACTACGCAGAGCATTTTCACACGTGCTACGGCCAAATTG
                                                                      6090
3'  CGGCCGCCCGCGATAATGGGTCTTCACGGAATGATGCGTCTCGTAAAAGTGTGCACGATGCCGGTTTAAC
    ---------------------------------- BGH13_B01b2-pDONR221 ----------------------------------

    A  G  G  R  Y  Y  P  E  V  P  Y  Y  A  E  H  F  H  T  C  Y  G  Q  I
    272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294

5'  ATTACGGTAACCGCTGGCAGGTACAAAACTGTGACTTGGTAGGGCTAAACGATTTAGCAACAGAACAAGA
                                                                      6160
3'  TAATGCCATTGGCGACCGTCCATGTTTTGACACTGAACCATCCCGATTTGCTAAATCGTTGTCTTGTTCT
    ---------------------------------- BGH13_B01b2-pDONR221 ----------------------------------

    D  Y  G  N  R  W  Q  V  Q  N  C  D  L  V  G  L  N  D  L  A  T  E  Q  D
    295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318

5'  CTATGTACGCACAAAAATAGCCAATTACTTAAACGACTTAACTAACCTTGGCGTAGCTGGCTTCCGCTTT
                                                                      6230
3'  GATACATGCGTGTTTTTATCGGTTAATGAATTTGCTGAATTGATTGGAACCGCATCGACCGAAGGCGAAA
    ---------------------------------- BGH13_B01b2-pDONR221 ----------------------------------

    Y  V  R  T  K  I  A  N  Y  L  N  D  L  T  N  L  G  V  A  G  F  R  F
    319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341

5'  GATGCTGCGAAACATATCCCCGTGGGTGACATTGCTGCAATTAAAGCAAAACTTATTGGCAACCCGTATA
                                                                      6300
3'  CTACGACGCTTTGTATAGGGGCACCCACTGTAACGACGTTAATTTCGTTTTGAATAACCGTTGGGCATAT
    ---------------------------------- BGH13_B01b2-pDONR221 ----------------------------------

    D  A  A  K  H  I  P  V  G  D  I  A  A  I  K  A  K  L  I  G  N  P  Y
    342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364
```

5' TATTCCAAGAAGTGATAGGTGCGCCGGGCGAGCCCATCCAACCATCAGAATATACCTACTTAGCAGACGT 6370
3' ATAAGGTTCTTCACTATCCACGCGGCCCGCTCGGGTAGGTTGGTAGTCTTATATGGATGAATCGTCTGCA
BGH13_B01b2-pDONR221
I F Q E V I G A P G E P I Q P S E Y T Y L A D V
365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388

5' AACAGAGTTTAACTTTACCAAATCGGTTAGCCATTTTTTTAAAGGGCGCGGACCAATTAAAGAGTTAAAA 6440
3' TTGTCTCAAATTGAAATGGTTTAGCCAATCGGTAAAAAAATTTCCCGCGCCTGGTTAATTTCTCAATTTT
BGH13_B01b2-pDONR221
T E F N F T K S V S H F F K G R G P I K E L K
389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411

5' AATATTGGTACTTGGGGCGGTTGGGTTGCAAGTGCAGACGCCGTAACCTTTGTTGCCAACCACGATAACC 6510
3' TTATAACCATGAACCCCGCCAACCCAACGTTCACGTCTGCGGCATTGGAAACAACGGTTGGTGCTATTGG
BGH13_B01b2-pDONR221
N I G T W G G W V A S A D A V T F V A N H D N
412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434

5' AGCGCCAGGATACAAACAACACCATTACCCATAAAGATGGTGATAACCGCAATACTATGGCGCACGTATT 6580
3' TCGCGGTCCTATGTTTGTTGTGGTAATGGGTATTTCTACCACTATTGGCGTTATGATACCGCGTGCATAA
BGH13_B01b2-pDONR221
Q R Q D T N N T I T H K D G D N R N T M A H V F
435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458

5' TATGCTGGGCTGGCCCTATGGGTACCCTAAAGTAATGTCTAGTTATGAGTGGTACGATCACGACCAAGGC 6650
3' ATACGACCCGACCGGGATACCCATGGGATTTCATTACAGATCAATACTCACCATGCTAGTGCTGGTTCCG
BGH13_B01b2-pDONR221
M L G W P Y G Y P K V M S S Y E W Y D H D Q G
459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481

5' CCTCCCGCCCACGGTGCTAGCAGCTGTAACAGCGGCTGGTTATGTGAACACCGCACGCCAGCTATCGCCA 6720
3' GGAGGGCGGGTGCCACGATCGTCGACATTGTCGCCGACCAATACACTTGTGGCGTGCGGTCGATAGCGGT
BGH13_B01b2-pDONR221
P P A H G A S S C N S G W L C E H R T P A I A
482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504

5' ATATGGTTGCTTTTCGCAATTTCACTGCAGACGAATTTCGCGTAACCAACTATTGGGATAACGGCAACAA 6790
3' TATACCAACGAAAAGCGTTAAAGTGACGTCTGCTTAAAGCGCATTGGTTGATAACCCTATTGCCGTTGTT

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N M V A F R N F T A D E F R V T N Y W D N G N N
505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528

5' TCAAATAGCTTGGGGCCGAGGCGGCAAAGGGTTTGTAGTTATAAATATGGAAGGCAACAATTTAACCCGT 6860
3' AGTTTATCGAACCCCGGCTCCGCCGTTTCCCAAACATCAATATTTATACCTTCCGTTGTTAAATTGGGCA

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Q I A W G R G G K G F V V I N M E G N N L T R
529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551

5' ACATTTCAAACAGGGATGCCCGCTGGCCGTTATTGCGACATTATTTCGGGTAACTACAACGCGTCTAATA 6930
3' TGTAAAGTTTGTCCCTACGGGCGACCGGCAATAACGCTGTAATAAAGCCCATTGATGTTGCGCAGATTAT

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T F Q T G M P A G R Y C D I I S G N Y N A S N
552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574

5' AAAGTTGTTCTGGCAATATTATAGATGTAGATAACAATGGCAACGCAGTAATCACCGCTTACTACCGCAA 7000
3' TTTCAACAAGACCGTTATAATATCTACATCTATTGTTACCGTTGCGTCATTAGTGGCGAATGATGGCGTT

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K S C S G N I I D V D N N G N A V I T A Y Y R N
575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598

5' CGGTGCCGCAATTCATGTAGGCGCTATTGTTGAAGACTGTGACGGTACAGATTGCTGCACAAACTGCTAG 7070
3' GCCACGGCGTTAAGTACATCCGCGATAACAACTTCTGACACTGCCATGTCTAACGACGTGTTTGACGATC

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G A A I H V G A I V E D C D G T D C C T N C .
599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621

EcoICRI
SacI
BamHI SalI HindIII NotI
EagI

5' GACCCAGCTTTCTTGTACAAAGTGGTTGATatcggatccgaattcgagctccgtcgacaagcttgcggcc 7140
3' CTGGGTCGAAAGAACATGTTTCACCAACTAtagcctaggcttaagctcgaggcagctgttcgaacgccgg

attB2

BGH13...R221

PspXI
XhoI
AvaI
BmeT110I

5' gcactcgagcaccaccaccaccaccactgagatccggctgctaacaaagcccgaaaggaagctgagttgg 7210
3' cgtgagctcgtggtggtggtggtggtgactctaggccgacgattgtttcgggctttccttcgactcaacc

BlpI

5' ctgctgccaccgctgagcaataactagcataaccccttggggcctctaaacgggtcttgaggggttttt 7280
3' gacgacggtggcgactcgttattgatcgtattggggaaccccggagatttgcccagaactccccaaaaaa

T7 Terminator

5' gctgaaaggaggaactatatccggat 7306
3' cgactttcctccttgatataggccta