```
5'  ttcttgaagacgaaagggcctcgtgatacgcctatttttataggttaatgtcatgataataatggtttct
                                                                        70
3'  aagaacttctgctttcccggagcactatgcggataaaaatatccaattacagtactattattaccaaaga

          ZraI
            AatII
5'  tagacgtcaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatacatt
                                                                       140
3'  atctgcagtccaccgtgaaaagcccctttacacgcgccttggggataaacaaataaaaagatttatgtaa

5'  caaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtat
                                                                       210
3'  gtttatacataggcgagtactctgttattgggactatttacgaagttattataactttttccttctcata

5'  gagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcac
                                                                       280
3'  ctcataagttgtaaaggcacagcgggaataagggaaaaaacgccgtaaaacggaaggacaaaaacgagtg
                                Ap

5'  ccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactgg
                                                                       350
3'  ggtctttgcgaccactttcattttctacgacttctagtcaacccacgtgctcacccaatgtagcttgacc
                                Ap

5'  atctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaa
                                                                       420
3'  tagagttgtcgccattctaggaactctcaaaagcggggcttcttgcaaaaggttactactcgtgaaaatt
                                Ap

5'  agttctgctatgtggcgcggtattatcccgtgttgacgccgggcaagagcaactcggtcgccgcatacac
                                                                       490
3'  tcaagacgatacaccgcgccataatagggcacaactgcggcccgttctcgttgagccagcggcgtatgtg
                                Ap

                                ScaI
5'  tattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaa
                                                                       560
3'  ataagagtcttactgaaccaactcatgagtggtcagtgtcttttcgtagaatgcctaccgtactgtcatt
                                Ap

                                                                  PvuI
5'  gagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcgg
                                                                       630
3'  ctcttaatacgtcacgacggtattggtactcactattgtgacgccggttgaatgaagactgttgctagcc
                                Ap
```

```
5'  aggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaa
                                                                      700
3'  tcctggcttcctcgattggcgaaaaaacgtgttgtacccccctagtacattgagcggaactagcaaccctt
                                  Ap

                                                          PstI
5'  ccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgcagcaatggcaacaacgt
                                                                      770
3'  ggcctcgacttacttcggtatggtttgctgctcgcactgtggtgctacggacgtcgttaccgttgttgca
                                  Ap

5'  tgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggaggc
                                                                      840
3'  acgcgtttgataattgaccgcttgatgaatgagatcgaagggccgttgttaattatctgacctacctccg
                                  Ap

5'  ggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctgga
                                                                      910
3'  cctatttcaacgtcctggtgaagacgcgagccgggaaggccgaccgaccaaataacgactatttagacct
                                  Ap

                          BsaI
5'  gccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtag
                                                                      980
3'  cggccactcgcacccagagcgccatagtaacgtcgtgaccccggtctaccattcgggagggcatagcatc
                                  Ap

                      AhdI
5'  ttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctc
                                                                     1050
3'  aatagatgtgctgcccctcagtccgttgatacctacttgctttatctgtctagcgactctatccacggag
                                  Ap

5'  actgattaagcattggtaactgtcagaccaagtttactcatatatactttagattgatttaaaacttcat
                                                                     1120
3'  tgactaattcgtaaccattgacagtctggttcaaatgagtatatatgaaatctaactaaattttgaagta
                                  Ap

5'  ttttaatttaaaaggatctaggtgaagatccttttttgataatctcatgaccaaaatcccttaacgtgagt
                                                                     1190
3'  aaaattaaattttcctagatccacttctaggaaaaactattagagtactggttttagggaattgcactca

5'  tttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcg
                                                                     1260
3'  aaagcaaggtgactcgcagtctggggcatcttttctagtttcctagaagaactctaggaaaaaaagacgc
                                                  Ori
```

5' cgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagcta
1330
3' gcattagacgacgaacgtttgtttttttggtggcgatggtcgccaccaaacaaacggcctagttctcgat
Ori

5' ccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagc
1400
3' ggttgagaaaaaggcttccattgaccgaagtcgtctcgcgtctatggtttatgacaggaagatcacatcg
Ori

5' cgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttacc
1470
3' gcatcaatccggtggtgaagttcttgagacatcgtggcggatgtatggagcgagacgattaggacaatgg
Ori

5' agtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataag
1540
3' tcaccgacgacggtcaccgctattcagcacagaatggcccaacctgagttctgctatcaatggcctattc
Ori

5' gcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaac
1610
3' cgcgtcgccagcccgacttgccccccaagcacgtgtgtcgggtcgaacctcgcttgctggatgtggcttg
Ori

5' tgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatcc
1680
3' actctatggatgtcgcactcgatactctttcgcggtgcgaagggcttccctctttccgcctgtccatagg
Ori

5' ggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttat
1750
3' ccattcgccgtcccagccttgtcctctcgcgtgctccctcgaaggtcccccttttgcggaccatagaaata
Ori

5' agtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcc
1820
3' tcaggacagcccaaagcggtggagactgaactcgcagctaaaaacactacgagcagtccccccgcctcgg
Ori

PciI

5' tatggaaaaacgccagcaacgcggcctttttacggttcctggccttttgctggccttttgctcacatgtt
1890
3' ataccttttgcggtcgttgcgccggaaaaatgccaaggaccggaaaacgaccggaaaacgagtgtacaa
Ori

5' ctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgc
1960
3' gaaaggacgcaatagggactaagacacctattggcataatggcggaaactcactcgactatggcgagcg

```
                                                   SapI
                                                    |
5'  cgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcctgatgcggtattttc
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2030
3'  gcgtcggcttgctggctcgcgtcgctcagtcactcgctccttcgccttctcgcggactacgccataaaag

5'  tccttacgcatctgtgcggtatttcacaccgcatatatggtgcactctcagtacaatctgctctgatgcc
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2100
3'  aggaatgcgtagacacgccataaagtgtggcgtatataccacgtgagagtcatgttagacgagactacgg

                                           BsaAI    Tth111I
                                             |        |
5'  gcatagttaagccagtatacactccgctatcgctacgtgactgggtcatggctgcgccccgacacccgcc
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2170
3'  cgtatcaattcggtcatatgtgaggcgatagcgatgcactgacccagtaccgacgcggggctgtgggcgg

5'  aacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgtc
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2240
3'  ttgtgggcgactgcgcgggactgcccgaacagacgagggccgtaggcgaatgtctgttcgacactggcag

5'  tccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgaggcagctgcggtaaagct
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2310
3'  aggccctcgacgtacacagtctccaaaagtggcagtagtggctttgcgcgctccgtcgacgccatttcga

5'  catcagcgtggtcgtgaagcgattcacagatgtctgcctgttcatccgcgtccagctcgttgagtttctc
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2380
3'  gtagtcgcaccagcacttcgctaagtgtctacagacggacaagtaggcgcaggtcgagcaactcaaagag

5'  cagaagcgttaatgtctggcttctgataaagcgggccatgttaagggcggtttttcctgtttggtcact
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2450
3'  gtcttcgcaattacagaccgaagactatttcgcccggtacaattcccgccaaaaaaggacaaaccagtga

5'  gatgcctccgtgtaagggggatttctgttcatgggggtaatgataccgatgaaacgagagaggatgctca
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2520
3'  ctacggaggcacattccccctaaagacaagtacccccattactatggctactttgctctctcctacgagt

5'  cgatacgggttactgatgatgaacatgcccggttactggaacgttgtgagggtaaacaactggcggtatg
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2590
3'  gctatgcccaatgactactacttgtacgggccaatgaccttgcaacactcccatttgttgaccgccatac

5'  gatgcggcgggaccagagaaaaatcactcagggtcaatgccagcgcttcgttaatacagatgtaggtgtt
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2660
3'  ctacgccgccctggtctctttttagtgagtcccagttacggtcgcgaagcaattatgtctacatccacaa

5'  ccacagggtagccagcagcatcctgcgatgcagatccggaacataatggtgcagggcgctgacttccgcg
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2730
3'  ggtgtcccatcggtcgtcgtaggacgctacgtctaggccttgtattaccacgtcccgcgactgaaggcgc

5'  tttccagactttacgaaacacggaaaccgaagaccattcatgttgttgctcaggtcgcagacgttttgca
    +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++  2800
3'  aaaggtctgaaatgctttgtgcctttggcttctggtaagtacaacaacgagtccagcgtctgcaaaacgt
```

```
5'   gcagcagtcgcttcacgttcgctcgcgtatcggtgattcattctgctaaccagtaaggcaaccccgccag
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2870
3'   cgtcgtcagcgaagtgcaagcgagcgcatagccactaagtaagacgattggtcattccgttggggcggtc
o
                                          FspAI        MscI
o                                         |            |
5'   cctagccgggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgcccgaga
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2940
3'   ggatcggcccaggagttgctgtcctcgtgctagtacgcgtgggcaccggtcctgggttgcgacgggctct
o
5'   tgcgccgcgtgcggctgctggagatggcggacgcgatggatatgttctgccaagggttggtttgcgcatt
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3010
3'   acgcggcgcacgccgacgacctctaccgcctgcgctacctatacaagacggttcccaaccaaacgcgtaa
o
5'   cacagttctccgcaagaattgattggctccaattcttggagtggtgaatccgttagcgaggtgccgccgg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3080
3'   gtgtcaagaggcgttcttaactaaccgaggttaagaacctcaccacttaggcaatcgctccacggcggcc
o
5'   cttccattcaggtcgaggtggcccggctccatgcaccgcgacgcaacgcggggaggcagacaaggtatag
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3150
3'   gaaggtaagtccagctccaccgggccgaggtacgtggcgctgcgttgcgcccctccgtctgttccatatc
o
5'   ggcggcgcctacaatccatgccaacccgttccatgtgctcgccgaggcggcataaatcgccgtgacgatc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3220
3'   ccgccgcggatgttaggtacggttgggcaaggtacacgagcggctccgccgtatttagcggcactgctag
o
5'   agcggtccagtgatcgaagttaggctggtaagagccgcgagcgatccttgaagctgtccctgatggtcgt
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3290
3'   tcgccaggtcactagcttcaatccgaccattctcggcgctcgctaggaacttcgacagggactaccagca
o
5'   catctacctgcctggacagcatggcctgcaacgcgggcatcccgatgccgccggaagcgagaagaatcat
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3360
3'   gtagatggacggacctgtcgtaccggacgttgcgcccgtagggctacggcggccttcgctcttcttagta
o
                                     NruI                                EagI
o                                    |                                   |
5'   aatggggaaggccatccagcctcgcgtcgcgaacgccagcaagacgtagcccagcgcgtcggccgccatg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3430
3'   ttacccttccggtaggtcggagcgcagcgcttgcggtcgttctgcatcgggtcgcgcagccggcggtac
o
5'   ccggcgataatggcctgcttctcgccgaaacgtttggtggcgggaccagtgacgaaggcttgagcgaggg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3500
3'   ggccgctattaccggacgaagagcggctttgcaaaccaccgccctggtcactgcttccgaactcgctccc
o
5'   cgtgcaagattccgaataccgcaagcgacaggccgatcatcgtcgcgctccagcgaaagcggtcctcgcc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3570
3'   gcacgttctaaggcttatggcgttcgctgtccggctagtagcagcgcgaggtcgctttcgccaggagcgg
o
5'   gaaaatgacccagagcgctgccggcacctgtcctacgagttgcatgataaagaagacagtcataagtgcg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3640
3'   cttttactgggtctcgcgacggccgtggacaggatgctcaacgtactatttcttctgtcagtattcacgc
o
```

PshAI

5' gcgacgatagtcatgccccgcgcccaccggaaggagctgactgggttgaaggctctcaagggcatcggtc 3710
3' cgctgctatcagtacggggcgcgggtggccttcctcgactgacccaacttccgagagttcccgtagccag

5' gagatcccggtgcctaatgagtgagctaacttacattaattgcgttgcgctcactgcccgctttccagtc 3780
3' ctctagggccacggattactcactcgattgaatgtaattaacgcaacgcgagtgacgggcgaaaggtcag

Lac I

5' gggaaacctgtcgtgccagctgcattaatgaatcggccaacgcgcggggagaggcggtttgcgtattggg 3850
3' ccctttggacagcacggtcgacgtaattacttagccggttgcgcgcccctctccgccaaacgcataaccc

Lac I

5' cgccagggtggtttttctttcaccagtgagacgggcaacagctgattgcccttcaccgcctggccctga 3920
3' gcggtcccaccaaaaagaaaagtggtcactctgcccgttgtcgactaacgggaagtggcggaccgggact

Lac I

HpaI

5' gagagttgcagcaagcggtccacgctggtttgccccagcaggcgaaaatcctgtttgatggtggttaacg 3990
3' ctctcaacgtcgttcgccaggtgcgaccaaacggggtcgtccgcttttaggacaaactaccaccaattgc

Lac I

5' gcgggatataacatgagctgtcttcggtatcgtcgtatcccactaccgagatatccgcaccaacgcgcag 4060
3' cgccctatattgtactcgacagaagccatagcagcatagggtgatggctctataggcgtggttgcgcgtc

Lac I

5' cccggactcggtaatggcgcgcattgcgcccagcgccatctgatcgttggcaaccagcatcgcagtggga 4130
3' gggcctgagccattaccgcgcgtaacgcgggtcgcggtagactagcaaccgttggtcgtagcgtcaccct

Lac I

5' acgatgccctcattcagcatttgcatggtttgttgaaaaccggacatggcactccagtcgccttcccgtt 4200
3' tgctacgggagtaagtcgtaaacgtaccaaacaacttttggcctgtaccgtgaggtcagcggaagggcaa

Lac I

5' ccgctatcggctgaatttgattgcgagtgagatatttatgccagccagccagacgcagacgcgccgagac 4270
3' ggcgatagccgacttaaactaacgctcactctataaatacggtcggtcggtctgcgtctgcgcggctctg

Lac I

PspOMI
ApaI

5' agaacttaatgggcccgctaacagcgcgatttgctggtgacccaatgcgaccagatgctccacgcccagt
3' tcttgaattacccgggcgattgtcgcgctaaacgaccactgggttacgctggtctacgaggtgcgggtca 4340
Lac I

5' cgcgtaccgtcttcatgggagaaaataatactgttgatgggtgtctggtcagagacatcaagaaataacg
3' gcgcatggcagaagtaccctcttttattatgacaactacccacagaccagtctctgtagttctttattgc 4410
Lac I

5' ccggaacattagtgcaggcagcttccacagcaatggcatcctggtcatccagcggatagttaatgatcag
3' ggccttgtaatcacgtccgtcgaaggtgtcgttaccgtaggaccagtaggtcgcctatcaattactagtc 4480
Lac I

MluI

5' cccactgacgcgttgcgcgagaagattgtgcaccgccgctttacaggcttcgacgccgcttcgttctacc
3' gggtgactgcgcaacgcgctcttctaacacgtggcggcgaaatgtccgaagctgcggcgaagcaagatgg 4550
Lac I

5' atcgacaccaccacgctggcacccagttgatcggcgcgagatttaatcgccgcgacaatttgcgacggcg
3' tagctgtggtggtgcgaccgtgggtcaactagccgcgctctaaattagcggcgctgttaaacgctgccgc 4620
Lac I

5' cgtgcagggccagactggaggtggcaacgccaatcagcaacgactgtttgcccgccagttgttgtgccac
3' gcacgtcccggtctgacctccaccgttgcggttagtcgttgctgacaaacgggcggtcaacaacacggtg 4690
Lac I

5' gcggttgggaatgtaattcagctccgccatcgccgcttccactttttcccgcgttttcgcagaaacgtgg
3' cgccaacccttacattaagtcgaggcggtagcggcgaaggtgaaaaagggcgcaaaagcgtctttgcacc 4760
Lac I

5' ctggcctggttcaccacgcgggaaacggtctgataagagacaccggcatactctgcgacatcgtataacg
3' gaccggaccaagtggtgcgccctttgccagactattctctgtggccgtatgagacgctgtagcatattgc 4830

5' ttactggtttcacattcaccaccctgaattgactctcttccgggcgctatcatgccataccgcgaaaggt
3' aatgaccaaagtgtaagtggtgggacttaactgagagaaggcccgcgatagtacggtatggcgctttcca 4900

5' tttgcgccattcgatggtgtccgggatctcgacgctctcccttatgcgactcctgcattaggaagcagcc
3' aaacgcggtaagctaccacaggccctagagctgcgagagggaatacgctgaggacgtaatccttcgtcgg 4970

```
5'  cagtagtaggttgaggccgttgagcaccgccgccgcaaggaatggtgcatgcaaggagatggcgcccaac
                                                                      5040
3'  gtcatcatccaactccggcaactcgtggcggcggcgttccttaccacgtacgttcctctaccgcgggttg

5'  agtcccccggccacggggcctgccaccatacccacgcccgaaacaagcgctcatgagcccgaagtggcgag
                                                                      5110
3'  tcagggggccggtgccccggacggtggtatgggtgcggctttgttcgcgagtactcgggcttcaccgctc

                                                                SgrAI
5'  cccgatcttccccatcggtgatgtcggcgatataggcgccagcaaccgcacctgtggcgccggtgatgcc
                                                                      5180
3'  gggctagaaggggtagccactacagccgctatatccgcggtcgttggcgtggacaccgcggccactacgg

                                        BglII
5'  ggccacgatgcgtccggcgtagaggatcgagatctcgatcccgcgaaattaatacgactcactatagggg
                                                                      5250
3'  ccggtgctacgcaggccgcatctcctagctctagagctagggcgctttaattatgctgagtgatatcccc
                                                        T7 Promoter

                                XbaI                    BarI
5'  aattgtgagcggataacaattcccctctagaaataattttgtttaactttaagaaggagatataccatgg
                                                                      5320
3'  ttaacactcgcctattgttaaggggagatctttattaaaacaaattgaaattcttcctctatatggtacc
    Lac O

                                                                    M
                                                                    1
            BarI'
5'  gccatcatcatcatcatcatcatcatcatcacagcagcggccatatcgaaggtcgtcatatgctcgaATC
                                                                      5390
3'  cggtagtagtagtagtagtagtagtagtgtcgtcgccggtatagcttccagcagtatacgagctTAG
    His10-tag                                   X-factor

    G   H   H   H   H   H   H   H   H   H   H   S   S   G   H   I   E   G   R   H   M   L   E   S
    2   3   4   5   6   7   8   9   10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25
5'  AACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttcaaggcACGACAACCCCCTGGTGGCGC
                                                                      5460
3'  TTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagttccgTGCTGTTGGGGGACCACCGCG
    attB1                   TEV site                BGH13_B04a
    BGH13_B04a-pDONR221

    T   S   L   Y   K   K   A   G   S   E   N   L   Y   F   Q   G   T   T   T   P   W   W   R
    26  27  28  29  30  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45  46  47  48
```

```
5'  GGCGCGGTTATTTATCAGGTATACCCCCGCAGTTTGATGGATACCAATGGCGATGGCATTGGTGATATTC
                                                                      5530
3'  CCGCGCCAATAAATAGTCCATATGGGGGCGTCAAACTACCTATGGTTACCGCTACCGTAACCACTATAAG
```

BGH13_B04a

BGH13_B04a-pDONR221

G	A	V	I	Y	Q	V	Y	P	R	S	L	M	D	T	N	G	D	G	I	G	D	I
49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71

```
5'  CCGGCATTATCAAAAAGCTCGACTACATCGCCAGCCTGGGTGTGGACGCCATTTGGGTATCGCCATTTTT
                                                                      5600
3'  GGCCGTAATAGTTTTTCGAGCTGATGTAGCGGTCGGACCCACACCTGCGGTAAACCCATAGCGGTAAAAA
```

BGH13_B04a

BGH13_B04a-pDONR221

P	G	I	I	K	K	L	D	Y	I	A	S	L	G	V	D	A	I	W	V	S	P	F	F
72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95

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5'  CAAGTCGCCGATGAAAGACTTCGGTTACGACATTAGTGACTATCGGGATATTGATCCCATCTTTGGAACC
                                                                      5670
3'  GTTCAGCGGCTACTTTCTGAAGCCAATGCTGTAATCACTGATAGCCCTATAACTAGGGTAGAAACCTTGG
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K	S	P	M	K	D	F	G	Y	D	I	S	D	Y	R	D	I	D	P	I	F	G	T
96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118

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5'  CTGAGTGATTTTGATGAGCTGATCAGCAAAGCCCATGCGCGCAATATCAAGATTATTATCGATCAGGTGC
                                                                      5740
3'  GACTCACTAAAACTACTCGACTAGTCGTTTCGGGTACGCGCGTTATAGTTCTAATAATAGCTAGTCCACG
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L	S	D	F	D	E	L	I	S	K	A	H	A	R	N	I	K	I	I	I	D	Q	V
119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141

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5'  TCAGCCATACCTCGGACCAGCATGCCTGGTTTTTGGAGAGCCGCGAGTCGCGCGACAACCCGAAAGCTGA
                                                                      5810
3'  AGTCGGTATGGAGCCTGGTCGTACGGACCAAAAACCTCTCGGCGCTCAGCGCGCTGTTGGGCTTTCGACT
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L	S	H	T	S	D	Q	H	A	W	F	L	E	S	R	E	S	R	D	N	P	K	A	D
142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165

MfeI

5' TTGGTATGTGTGGGTAGATCCTCAACCCGACGGAACCCCCCCCAACAATTGGTTGGCAATTTTTGGGGGC 5880
3' AACCATACACACCCATCTAGGAGTTGGGCTGCCTTGGGGGGGGTTGTTAACCAACCGTTAAAAACCCCCG

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W Y V W V D P Q P D G T P P N N W L A I F G G
166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188

5' TCTGCCTGGGAGTGGGAGCCGCGTCGTTGTCAATATTACCTGCATAACTTTTTGCGTACCCAGCCAGATT 5950
3' AGACGGACCCTCACCCTCGGCGCAGCAACAGTTATAATGGACGTATTGAAAAACGCATGGGTCGGTCTAA

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S A W E W E P R R C Q Y Y L H N F L R T Q P D
189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211

SwaI AlaI' AlaI

5' TAAATTACCACTGTCCGCAAGTGCGCGAGCAAATTTTGCAGGAAGTGGAGTTCTGGTTGAAACGCGGCGT 6020
3' ATTTAATGGTGACAGGCGTTCACGCGCTCGTTTAAAACGTCCTTCACCTCAAGACCAACTTTGCGCCGCA

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L N Y H C P Q V R E Q I L Q E V E F W L K R G V
212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235

5' GGATGGTTTGCGCCTTGATGCCATTAACTTCTGCTTTCACGATAAGGAATTGCGTTCCAATCCTGCCAAG 6090
3' CCTACCAAACGCGGAACTACGGTAATTGAAGACGAAAGTGCTATTCCTTAACGCAAGGTTAGGACGGTTC

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D G L R L D A I N F C F H D K E L R S N P A K
236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258

5' CCGGAAGCAGAGCGTAAGGGGCGAGGCTTTAAGGAAGACAACCCCTACGCATTCCAGAAACACATATTTG 6160
3' GGCCTTCGTCTCGCATTCCCCGCTCCGAAATTCCTTCTGTTGGGGATGCGTAAGGTCTTTGTGTATAAAC

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P E A E R K G R G F K E D N P Y A F Q K H I F
259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281

XmaI SmaI

5' ATAATACCCGCCCGGAAAACCTGGCTTTCCTGGAGTCCTTGCGCCAGTTAATGGATCGTTATCCCGGGAC 6230
3' TATTATGGGCGGGCCTTTTGGACCGAAAGGACCTCAGGAACGCGGTCAATTACCTAGCAATAGGGCCCTG

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D N T R P E N L A F L E S L R Q L M D R Y P G T
282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305

5' GGTAACCCTCGGTGAAATTTCAGCCGAGGACTCCCTGAAAACCATGGCGGAATACACCTCCGGTAACGAC 6300
3' CCATTGGGAGCCACTTTAAAGTCGGCTCCTGAGGGACTTTTGGTACCGCCTTATGTGGAGGCCATTGCTG

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V T L G E I S A E D S L K T M A E Y T S G N D
306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328

5' CGTTTGCATATGGCGTATAGCTTTGAACTGCTGGTCGATAAGTTATCCGGCAACTATATCCGCAATACGG 6370
3' GCAAACGTATACCGCATATCGAAACTTGACGACCAGCTATTCAATAGGCCGTTGATATAGGCGTTATGCC

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R L H M A Y S F E L L V D K L S G N Y I R N T
329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351

BbvCI DraIII

5' TGGAAACCCTGGAAGCCAATTTGGCTGAGGGCTGGCCCTGTTGGTCTATAGGCAACCACGATGTGGTGCG 6440
3' ACCTTTGGGACCTTCGGTTAAACCGACTCCCGACCGGGACAACCAGATATCCGTTGGTGCTACACCACGC

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V E T L E A N L A E G W P C W S I G N H D V V R
352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375

5' ATTTATGTCGCGTTGGGGCGGTGAGGCACAATCCCCCGAACTCGCCAAAACCCTCAATGCCATGCTGCTT 6510
3' TAAATACAGCGCAACCCCGCCACTCCGTGTTAGGGGGCTTGAGCGGTTTTGGGAGTTACGGTACGACGAA

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F M S R W G G E A Q S P E L A K T L N A M L L
376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398

5' TCATTGCGCGGCAGTGTGTGTAGCTATCAGGGGGAAGAACTGGGTTTGACAGAAGCGGATATCCAGCAGC 6580
3' AGTAACGCGCCGTCACACACATCGATAGTCCCCCTTCTTGACCCAAACTGTCTTCGCCTATAGGTCGTCG

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S L R G S V C S Y Q G E E L G L T E A D I Q Q
399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421

5' ATGAGTTGCAAGATCCCTATGGTATTACCTTCTGGCCTCGCTTCAAGGGGCGCGATGGATGTAGAACGCC 6650
3' TACTCAACGTTCTAGGGATACCATAATGGAAGACCGGAGCGAAGTTCCCCGCGCTACCTACATCTTGCGG

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H E L Q D P Y G I T F W P R F K G R D G C R T P
422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445

PasI

5' AATGCCCTGGGACTCATCCCTGGAATACGCGGGATTTTCCAGTGCAAAACCATGGCTACCCGTATCGGCA 6720
3' TTACGGGACCCTGAGTAGGGACCTTATGCGCCCTAAAAGGTCACGTTTTGGTACCGATGGGCATAGCCGT

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M P W D S S L E Y A G F S S A K P W L P V S A
446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468

5' AAACACCTGGCGCTCTCCATCGATAAACAGGAAAGCAATCAGGCATCTGTGCTGAACGCCTATCGCCATT 6790
3' TTTGTGGACCGCGAGAGGTAGCTATTTGTCCTTTCGTTAGTCCGTAGACACGACTTGCGGATAGCGGTAA

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K H L A L S I D K Q E S N Q A S V L N A Y R H
469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491

5' TTATGCAGTGGCGTAAAACGCAAGCTGCCCTGCGCTTTGGTGGCATTGAATTTCTCTCCAGTAGTGAATC 6860
3' AATACGTCACCGCATTTTGCGTTCGACGGGACGCGAAACCACCGTAACTTAAAGAGAGGTCATCACTTAG

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F M Q W R K T Q A A L R F G G I E F L S S S E S
492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515

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5'  TCATTTGGTGTTTGTTCGTCGCCATGGCAATGAGGCGCTATTGTGTGGATTTAATTTCAGTGCCCAGCCT
                                                                          6930
3'  AGTAAACCACAAACAAGCAGCGGTACCGTTACTCCGCGATAACACACCTAAATTAAAGTCACGGGTCGGA
    BGH13_B04a
    BGH13_B04a-pDONR221

    H  L  V  F  V  R  R  H  G  N  E  A  L  L  C  G  F  N  F  S  A  Q  P
    516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538

5'  GCCGTTATTGAGTTGGGACAAGTGTTGGGCCTGGAGTCAATAACCGGCCATGGTTTTGCAACGCAATCCT
                                                                          7000
3'  CGGCAATAACTCAACCCTGTTCACAACCCGGACCTCAGTTATTGGCCGGTACCAAAACGTTGCGTTAGGA
    BGH13_B04a
    BGH13_B04a-pDONR221

    A  V  I  E  L  G  Q  V  L  G  L  E  S  I  T  G  H  G  F  A  T  Q  S
    539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561

5'  CCCGGTCAAACAATATTGGTTTGCCGGGGTATAGCGCGTTTTTTGCCAGCCTTGTCTAGGACCCAGCTTT
                                                                          7070
3'  GGGCCAGTTTGTTATAACCAAACGGCCCCATATCGCGCAAAAAACGGTCGGAACAGATCCTGGGTCGAAA
    BGH13_B04a                                                  attB2
    BGH13_B04a-pDONR221

    S  R  S  N  N  I  G  L  P  G  Y  S  A  F  F  A  S  L  V  .
    562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581

                                BamHI
5'  CTTGTACAAAGTGGTTGATtcgaggatccggctgctaacaaagcccgaaaggaagctgagttggctgctg
                                                                          7140
3'  GAACATGTTTCACCAACTAagctcctaggccgacgattgtttcgggctttccttcgactcaaccgacgac
    attB2

5'  ccaccgctgagcaataactagcataaccccttggggcctctaaacgggtcttgaggggttttttgctgaa
                                                                          7210
3'  ggtggcgactcgttattgatcgtattggggaaccccggagatttgcccagaactccccaaaaaacgactt
    T7 Terminator

5'  aggaggaactatatccggatatcccgcaagaggcccggcagtaccggcataaccaagcctatgcctacag
                                                                          7280
3'  tcctccttgatataggcctatagggcgttctccgggccgtcatggccgtattggttcggatacggatgtc

5'  catccagggtgacggtgccgaggatgacgatgagcgcattgttagatttcatacacggtgcctgactgcg
                                                                          7350
3'  gtaggtcccactgccacggctcctactgctactcgcgtaacaatctaaagtatgtgccacggactgacgc

                                        HindIII                   EcoRI
5'  ttagcaatttaactgtgataaactaccgcattaaagcttatcgatgataagctgtcaaacatgagaa
                                                                          7417
3'  aatcgttaaattgacactatttgatggcgtaatttcgaatagctactattcgacagtttgtactctt
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