BGT04_T02a-pET16-DEST

```
5'  ttcttgaagacgaaagggcctcgtgatacgcctatttttataggttaatgtcatgataataatggtttct
    +++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|  70
3'  aagaacttctgctttcccggagcactatgcggataaaaatatccaattacagtactattattaccaaaga

        ZraI
          AatII
5'  tagacgtcaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatacatt
    +++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++| 140
3'  atctgcagtccaccgtgaaaagcccctttacacgcgccttggggataaacaaataaaaagatttatgtaa

                                                                SspI
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'  aggaccgaaggagctaaccgctttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaa
    +++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|  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
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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'  atacctttttgcggtcgttgcgccggaaaaatgccaaggaccggaaaacgaccggaaaacgagtgtacaa
    ==========================================>
    ---------------Ori---------------

5'  ctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgc
    +++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|++++++|  1960
3'  gaaaggacgcaatagggactaagacacctattggcataatggcggaaactcactcgactatggcgagcg
```

SapI

5' cgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcctgatgcggtattttc
3' gcgtcggcttgctggctcgcgtcgctcagtcactcgctccttcgccttctcgcggactacgccataaaag 2030

5' tccttacgcatctgtgcggtatttcacaccgcatatatggtgcactctcagtacaatctgctctgatgcc
3' aggaatgcgtagacacgccataaagtgtggcgtatataccacgtgagagtcatgttagacgagactacgg 2100

BstZ17I BsaAI

5' gcatagttaagccagtatacactccgctatcgctacgtgactgggtcatggctgcgccccgacacccgcc
3' cgtatcaattcggtcatatgtgaggcgatagcgatgcactgacccagtaccgacgcggggctgtgggcgg 2170

5' aacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgtc
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3' ctacgccgccctggtctctttttagtgagtcccagttacggtcgcgaagcaattatgtctacatccacaa 2660

5' ccacagggtagccagcagcatcctgcgatgcagatccggaacataatggtgcagggcgctgacttccgcg
3' ggtgtcccatcggtcgtcgtaggacgctacgtctaggccttgtattaccacgtcccgcgactgaaggcgc 2730

5' tttccagactttacgaaacacggaaaccgaagaccattcatgttgttgctcaggtcgcagacgttttgca
3' aaaggtctgaaatgctttgtgcctttggcttctggtaagtacaacaacgagtccagcgtctgcaaaacgt 2800

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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
                                                                  EagI
o                                                                 |
5'   aatggggaaggccatccagcctcgcgtcgcgaacgccagcaagacgtagcccagcgcgtcggccgccatg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3430
3'   ttacccttccggtaggtcggagcgcagcgcttgcggtcgttctgcatcgggtcgcgcagccggcggtac
o
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o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3500
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5'   cgtgcaagattccgaataccgcaagcgacaggccgatcatcgtcgcgctccagcgaaagcggtcctcgcc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3570
3'   gcacgttctaaggcttatggcgttcgctgtccggctagtagcagcgcgaggtcgctttcgccaggagcgg
o
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o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3640
3'   cttttactgggtctcgcgacggccgtggacaggatgctcaacgtactatttcttctgtcagtattcacgc
o
```

PshAI

5' gcgacgatagtcatgccccgcgcccaccggaaggagctgactgggttgaaggctctcaagggcatcggtc
3' cgctgctatcagtacggggcgcgggtggccttcctcgactgacccaacttccgagagttcccgtagccag 3710

5' gagatcccggtgcctaatgagtgagctaacttacattaattgcgttgcgctcactgcccgctttccagtc
3' ctctagggccacggattactcactcgattgaatgtaattaacgcaacgcgagtgacgggcgaaaggtcag 3780

Lac I

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3' ccctttggacagcacggtcgacgtaattacttagccggttgcgcgcccctctccgccaaacgcataaccc 3850

Lac I

5' cgccagggtggtttttctttcaccagtgagacgggcaacagctgattgcccttcaccgcctggccctga
3' gcggtcccaccaaaaagaaaagtggtcactctgcccgttgtcgactaacgggaagtggcggaccgggact 3920

Lac I

HpaI

5' gagagttgcagcaagcggtccacgctggtttgccccagcaggcgaaaatcctgtttgatggtggttaacg
3' ctctcaacgtcgttcgccaggtgcgaccaaacggggtcgtccgcttttaggacaaactaccaccaattgc 3990

Lac I

5' gcgggatataacatgagctgtcttcggtatcgtcgtatcccactaccgagatatccgcaccaacgcgcag
3' cgccctatattgtactcgacagaagccatagcagcatagggtgatggctctataggcgtggttgcgcgtc 4060

Lac I

BssHII

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Lac I

5' acgatgccctcattcagcatttgcatggtttgttgaaaaccggacatggcactccagtcgccttcccgtt
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Lac I

5' ccgctatcggctgaatttgattgcgagtgagatatttatgccagccagccagacgcagacgcgccgagac
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Lac I

BstEII

5' agaacttaatgggcccgctaacagcgcgatttgctggtgacccaatgcgaccagatgctccacgcccagt 4340
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Lac I

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Lac I

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Lac I

MluI

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Lac I

5' atcgacaccaccacgctggcacccagttgatcggcgcgagatttaatcgccgcgacaatttgcgacggcg 4620
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Lac I

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Lac I

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Lac I

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EcoNI

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3' aaacgcggtaagctaccacaggccctagagctgcgagagggaatacgctgaggacgtaatccttcgtcgg

```
5'  cagtagtaggttgaggccgttgagcaccgccgccgcaaggaatggtgcatgcaaggagatggcgcccaac
                                                                          5040
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5'  agtcccccggccacggggcctgccaccatacccacgccgaaacaagcgctcatgagcccgaagtggcgag
                                                                          5110
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                                                                SgrAI
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                                                                          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'  AACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttcaaggcCGCGTAGCTATGATTTCCATG
                                                                          5460
3'  TTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagttccgGCGCATCGATACTAAAGGTAC
    attB1                   TEV site                BGT04_T02a
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    T  S  L  Y  K  K  A  G  S  E  N  L  Y  F  Q  G  R  V  A  M  I  S  M
    26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
```

5' CACACCTCTCCATTGCAGCAGCCCGGAACTGGTGATTCAGGCGGCATGAACGTCTACATTCTTTCGACCG 5530
3' GTGTGGAGAGGTAACGTCGTCGGGCCTTGACCACTAAGTCCGCCGTACTTGCAGATGTAAGAAAGCTGGC

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

NheI BmtI StuI

5' CGACTGAGCTAGCGAAACAGGGTATCGAGGTCGATATTTACACTCGTGCCACGAGGCCTTCTCAGGGTGA 5600
3' GCTGACTCGATCGCTTTGTCCCATAGCTCCAGCTATAAATGTGAGCACGGTGCTCCGGAAGAGTCCCACT

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

5' GATCGTGAGAGTAGCTGAGAATTTGCGGGTCATTAATATCGCTGCGGGGCCGTATGAGGGGCTTTCCAAA 5670
3' CTAGCACTCTCATCGACTCTTAAACGCCCAGTAATTATAGCGACGCCCCGGCATACTCCCCGAAAGGTTT

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I V R V A E N L R V I N I A A G P Y E G L S K
96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118

5' GAGGAGCTTCCTACTCAGTTGGCGGCGTTTACCGGCGGAATGTTGTCGTTTACGCGCCGGGAGAAGGTTA 5740
3' CTCCTCGAAGGATGAGTCAACCGCCGCAAATGGCCGCCTTACAACAGCAAATGCGCGGCCCTCTTCCAAT

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E E L P T Q L A A F T G G M L S F T R R E K V
119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141

5' CTTATGATCTGATCCATTCTCACTATTGGCTGTCTGGTCAGGTGGGGTGGTTGCTGCGCGATTTGTGGCG 5810
3' GAATACTAGACTAGGTAAGAGTGATAACCGACAGACCAGTCCACCCCACCAACGACGCGCTAAACACCGC

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T Y D L I H S H Y W L S G Q V G W L L R D L W R
142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165

5' GATTCCCCTTATTCATACGGCACACACTTTGGCGGCGGTGAAGAATTCTTATCGGGATGATTCGGACACT 5880
3' CTAAGGGGAATAAGTATGCCGTGTGTGAAACCGCCGCCACTTCTTAAGAATAGCCCTACTAAGCCTGTGA

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

5' CCGGAGTCGGAGGCGCGTCGCATTTGTGAGCAGCAGCTGGTGGATAACGCTGACGTGTTGGCGGTGAACA 5950
3' GGCCTCAGCCTCCGCGCAGCGTAAACACTCGTCGTCGACCACCTATTGCGACTGCACAACCGCCACTTGT

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

NsiI

5' CTCAGGAGGAGATGCAGGATTTGATGCATCACTACGATGCGGATCCGGATCGGATTTCTGTGGTGTCACC 6020
3' GAGTCCTCCTCTACGTCCTAAACTACGTAGTGATGCTACGCCTAGGCCTAGCCTAAAGACACCACAGTGG

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T Q E E M Q D L M H H Y D A D P D R I S V V S P
212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235

DraIII

5' GGGTGCGGACGTGGAACTTTATAGCCCTGGAAATGATCGCGCGACGGAACGTTCCCGTCGTGAGCTGGGC 6090
3' CCCACGCCTGCACCTTGAAATATCGGGACCTTTACTAGCGCGCTGCCTTGCAAGGGCAGCACTCGACCCG

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

CspCI CspCI' AarI

5' ATTCCGCTGCACACAAAGGTAGTGGCTTTTGTGGGTCGGTTGCAGCCGTTTAAGGGCCCGCAGGTGCTGA 6160
3' TAAGGCGACGTGTGTTTCCATCACCGAAAACACCCAGCCAACGTCGGCAAATTCCCGGGCGTCCACGACT

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

RsrII

5' TCAAGGCGGTTGCGGCGTTGTTTGATCGCGATCCGGACCGAAATCTGCGCGTCATTATTTGTGGCGGCCC 6230
3' AGTTCCGCCAACGCCGCAACAAACTAGCGCTAGGCCTGGCTTTAGACGCGCAGTAATAAACACCGCCGGG

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I K A V A A L F D R D P D R N L R V I I C G G P
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' TTCTGGTCCGAATGCGACACCGGATACCTATAGGCATATGGCAGAGGAACTGGGCGTCGAAAAGCGAATT 6300
3' AAGACCAGGCTTACGCTGTGGCCTATGGATATCCGTATACCGTCTCCTTGACCCGCAGCTTTTCGCTTAA

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

5' CGCTTTTTGGACCCGCGCCCGCCGAGCGAGCTAGTGGCCGTGTATCGGGCGGCGGACATCGTGGCCGTGC 6370
3' GCGAAAAACCTGGGCGCGGGCGGCTCGCTCGATCACCGGCACATAGCCCGCCGCCTGTAGCACCGGCACG

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

AgeI

5' CAAGTTTTAATGAGTCCTTCGGACTCGTCGCCATGGAGGCGCAAGCCAGCGGCACACCGGTCATTGCGGC 6440
3' GTTCAAAATTACTCAGGAAGCCTGAGCAGCGGTACCTCCGCGTTCGGTCGCCGTGTGGCCAGTAACGCCG

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

XmaI
SmaI

5' CCGGGTTGGCGGCCTGCCCATCGCAGTCGCGGAAGGGGAGACGGGATTGCTTGTCGACGGCCACTCCCCG 6510
3' GGCCCAACCGCCGGACGGGTAGCGTCAGCGCCTTCCCCTCTGCCCTAACGAACAGCTGCCGGTGAGGGGC

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R V G G L P I A V A E G E T G L L V D G H S P
376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398

```
5'  CATGCCTGGGCCGACGCCTTAGCCACACTCTTGGACGATGACGAAACGCGCATCAGAATGGGTGAAGACG
                                                                          6580
3'  GTACGGACCCGGCTGCGGAATCGGTGTGAGAACCTGCTACTGCTTTGCGCGTAGTCTTACCCACTTCTGC
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    H   A   W   A   D   A   L   A   T   L   L   D   D   D   E   T   R   I   R   M   G   E   D
    399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421

5'  CCGTCGAACACGCCAGAACATTCTCCTGGGCGGCCACCGCCGCACAGCTATCGTCGCTGTACAACGACGC
                                                                          6650
3'  GGCAGCTTGTGCGGTCTTGTAAGAGGACCCGCCGGTGGCGGCGTGTCGATAGCAGCGACATGTTGCTGCG
```

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    A   V   E   H   A   R   T   F   S   W   A   A   T   A   A   Q   L   S   S   L   Y   N   D   A
    422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445

5'  TATTGCCAACGAAAATGTCGACGGTGAAACGCATCACGGCTAGGACCCAGCTTTCTTGTACAAAGTGGTT
                                                                          6720
3'  ATAACGGTTGCTTTTACAGCTGCCACTTTGCGTAGTGCCGATCCTGGGTCGAAAGAACATGTTTCACCAA
```

BGT04_T02a attB2

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```
    I   A   N   E   N   V   D   G   E   T   H   H   G   .
    446 447 448 449 450 451 452 453 454 455 456 457 458 459

                                                      BlpI
5'  GATtcgaggatccggctgctaacaaagcccgaaaggaagctgagttggctgctgccaccgctgagcaata
                                                                          6790
3'  CTAagctcctaggccgacgattgtttcgggctttccttcgactcaaccgacgacggtggcgactcgttat
```

T7 Terminator

```
5'  actagcataaccccttggggcctctaaacgggtcttgaggggttttttgctgaaaggaggaactatatcc
                                                                          6860
3'  tgatcgtattggggaaccccggagatttgcccagaactccccaaaaaacgactttcctccttgatatagg
```

T7 Terminator

```
5'  ggatatcccgcaagaggcccggcagtaccggcataaccaagcctatgcctacagcatccagggtgacggt
                                                                          6930
3'  cctatagggcgttctccgggccgtcatggccgtattggttcggatacggatgtcgtaggtcccactgcca

5'  gccgaggatgacgatgagcgcattgttagatttcatacacggtgcctgactgcgttagcaatttaactgt
                                                                          7000
3'  cggctcctactgctactcgcgtaacaatctaaagtatgtgccacggactgacgcaatcgttaaattgaca

                        HindIII     ClaI
5'  gataaactaccgcattaaagcttatcgatgataagctgtcaaacatgagaa
                                                                          7051
3'  ctatttgatggcgtaatttcgaatagctactattcgacagtttgtactctt
```