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

            ZraI
o             AatII

5'  tagacgtcaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatacatt
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  140
3'  atctgcagtccaccgtgaaaagcccctttacacgcgccttggggataaacaaataaaaagatttatgtaa
o

5'  caaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtat
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  210
3'  gtttatacataggcgagtactctgttattgggactatttacgaagttattataactttttccttctcata
o

5'  gagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcac
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  280
3'  ctcataagttgtaaaggcacagcgggaataagggaaaaaacgccgtaaaacggaaggacaaaaacgagtg
o   ================================================================
                                   Ap
o

5'  ccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactgg
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  350
3'  ggtctttgcgaccactttcattttctacgacttctagtcaacccacgtgctcacccaatgtagcttgacc
o   ================================================================
                                   Ap
o

5'  atctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaa
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  420
3'  tagagttgtcgccattctaggaactctcaaaagcggggcttcttgcaaaaggttactactcgtgaaaatt
o   ================================================================
                                   Ap
o

5'  agttctgctatgtggcgcggtattatcccgtgttgacgccgggcaagagcaactcggtcgccgcatacac
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  490
3'  tcaagacgatacaccgcgccataatagggcacaactgcggcccgttctcgttgagccagcggcgtatgtg
o   ================================================================
                                   Ap
o

o                                 ScaI

5'  tattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaa
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  560
3'  ataagagtcttactgaaccaactcatgagtggtcagtgtcttttcgtagaatgcctaccgtactgtcatt
o   ================================================================
                                   Ap
o

5'  gagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcgg
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  630
3'  ctcttaatacgtcacgacggtattggtactcactattgtgacgccggttgaatgaagactgttgctagcc
o   ================================================================
                                   Ap
o

5'  aggaccgaaggagctaaccgctttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaa
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  700
3'  tcctggcttcctcgattggcgaaaaaacgtgttgtacccccctagtacattgagcggaactagcaaccctt
o   ================================================================
                                   Ap
o
```

PstI

5' ccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgcagcaatggcaacaacgt 770
3' ggcctcgacttacttcggtatggtttgctgctcgcactgtggtgctacggacgtcgttaccgttgttgca
Ap

5' tgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggaggc 840
3' acgcgtttgataattgaccgcttgatgaatgagatcgaagggccgttgttaattatctgacctacctccg
Ap

5' ggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctgga 910
3' cctatttcaacgtcctggtgaagacgcgagccgggaaggccgaccgaccaaataacgactatttagacct
Ap

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
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1400
3'  ggttgagaaaaaggcttccattgaccgaagtcgtctcgcgtctatggtttatgacaggaagatcacatcg
o   ==========================================================================
                                   Ori
o
5'  cgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttacc
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1470
3'  gcatcaatccggtggtgaagttcttgagacatcgtggcggatgtatggagcgagacgattaggacaatgg
o   ==========================================================================
                                   Ori
o
5'  agtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataag
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1540
3'  tcaccgacgacggtcaccgctattcagcacagaatggcccaacctgagttctgctatcaatggcctattc
o   ==========================================================================
                                   Ori
o
5'  gcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaac
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1610
3'  cgcgtcgccagcccgacttgccccccaagcacgtgtgtcgggtcgaacctcgcttgctggatgtggcttg
o   ==========================================================================
                                   Ori
o
5'  tgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatcc
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1680
3'  actctatggatgtcgcactcgatactctttcgcggtgcgaagggcttccctctttccgcctgtccatagg
o   ==========================================================================
                                   Ori
o
5'  ggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttat
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1750
3'  ccattcgccgtcccagccttgtcctctcgcgtgctccctcgaaggtcccccttttgcggaccatagaaata
o   ==========================================================================
                                   Ori
o
5'  agtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcc
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1820
3'  tcaggacagcccaaagcggtggagactgaactcgcagctaaaaacactacgagcagtcccccccgcctcgg
o   ==========================================================================
                                   Ori
o
                                                                   PciI
o                                                                  |
5'  tatggaaaaacgccagcaacgcggcctttttacggttcctggccttttgctggccttttgctcacatgtt
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1890
3'  atacctttttgcggtcgttgcgccggaaaaatgccaaggaccggaaaacgaccggaaaacgagtgtacaa
o   ==========>
                 Ori
o
5'  ctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgc
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1960
3'  gaaaggacgcaatagggactaagacacctattggcataatggcggaaactcactcgactatggcgagcg
o
                                          SapI
o                                         |
5'  cgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcctgatgcggtattttc
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2030
3'  gcgtcggcttgctggctcgcgtcgctcagtcactcgctccttcgccttctcgcggactacgccataaaag
o
```

```
5'   tccttacgcatctgtgcggtatttcacaccgcatatatggtgcactctcagtacaatctgctctgatgcc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2100
3'   aggaatgcgtagacacgccataaagtgtggcgtatataccacgtgagagtcatgttagacgagactacgg
o

                        BstZ17I                 BsaAI    Tth111I
o                       |                       |        |
5'   gcatagttaagccagtatacactccgctatcgctacgtgactgggtcatggctgcgccccgacacccgcc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2170
3'   cgtatcaattcggtcatatgtgaggcgatagcgatgcactgacccagtaccgacgcggggctgtgggcgg
o

5'   aacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgtc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2240
3'   ttgtgggcgactgcgcgggactgcccgaacagacgagggccgtaggcgaatgtctgttcgacactggcag
o

5'   tccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgaggcagctgcggtaaagct
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2310
3'   aggccctcgacgtacacagtctccaaaagtggcagtagtggctttgcgcgctccgtcgacgccatttcga
o

5'   catcagcgtggtcgtgaagcgattcacagatgtctgcctgttcatccgcgtccagctcgttgagtttctc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2380
3'   gtagtcgcaccagcacttcgctaagtgtctacagacggacaagtaggcgcaggtcgagcaactcaaagag
o

5'   cagaagcgttaatgtctggcttctgataaagcgggccatgttaagggcggtttttcctgtttggtcact
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2450
3'   gtcttcgcaattacagaccgaagactatttcgcccggtacaattcccgccaaaaaaggacaaaccagtga
o

5'   gatgcctccgtgtaagggggatttctgttcatgggggtaatgataccgatgaaacgagagaggatgctca
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2520
3'   ctacggaggcacattccccctaaagacaagtacccccattactatggctactttgctctctcctacgagt
o

5'   cgatacgggttactgatgatgaacatgcccggttactggaacgttgtgagggtaaacaactggcggtatg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2590
3'   gctatgcccaatgactactacttgtacgggccaatgaccttgcaacactcccatttgttgaccgccatac
o

5'   gatgcggcgggaccagagaaaaatcactcagggtcaatgccagcgcttcgttaatacagatgtaggtgtt
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2660
3'   ctacgccgccctggtctctttttagtgagtcccagttacggtcgcgaagcaattatgtctacatccacaa
o

5'   ccacagggtagccagcagcatcctgcgatgcagatccggaacataatggtgcagggcgctgacttccgcg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2730
3'   ggtgtcccatcggtcgtcgtaggacgctacgtctaggccttgtattaccacgtcccgcgactgaaggcgc
o

                                                        Bpu10I
o                                                       |
5'   tttccagactttacgaaacacggaaaccgaagaccattcatgttgttgctcaggtcgcagacgttttgca
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2800
3'   aaaggtctgaaatgctttgtgcctttggcttctggtaagtacaacaacgagtccagcgtctgcaaaacgt
o

5'   gcagcagtcgcttcacgttcgctcgcgtatcggtgattcattctgctaaccagtaaggcaaccccgccag
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2870
3'   cgtcgtcagcgaagtgcaagcgagcgcatagccactaagtaagacgattggtcattccgttggggcggtc
o
```

FspAI MscI

```
5'  cctagccgggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgcccgaga
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  2940
3'  ggatcggcccaggagttgctgtcctcgtgctagtacgcgtgggcaccggtcctgggttgcgacgggctct

5'  tgcgccgcgtgcggctgctggagatggcggacgcgatggatatgttctgccaagggttggtttgcgcatt
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3010
3'  acgcggcgcacgccgacgacctctaccgcctgcgctacctatacaagacggttcccaaccaaacgcgtaa

5'  cacagttctccgcaagaattgattggctccaattcttggagtggtgaatccgttagcgaggtgccgccgg
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3080
3'  gtgtcaagaggcgttcttaactaaccgaggttaagaacctcaccacttaggcaatcgctccacggcggcc

5'  cttccattcaggtcgaggtggcccggctccatgcaccgcgacgcaacgcggggaggcagacaaggtatag
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3150
3'  gaaggtaagtccagctccaccgggccgaggtacgtggcgctgcgttgcgcccctccgtctgttccatatc

5'  ggcggcgcctacaatccatgccaacccgttccatgtgctcgccgaggcggcataaatcgccgtgacgatc
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3220
3'  ccgccgcggatgttaggtacggttgggcaaggtacacgagcggctccgccgtatttagcggcactgctag

5'  agcggtccagtgatcgaagttaggctggtaagagccgcgagcgatccttgaagctgtccctgatggtcgt
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3290
3'  tcgccaggtcactagcttcaatccgaccattctcggcgctcgctaggaacttcgacagggactaccagca

5'  catctacctgcctggacagcatggcctgcaacgcgggcatcccgatgccgccggaagcgagaagaatcat
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3360
3'  gtagatggacggacctgtcgtaccggacgttgcgcccgtagggctacggcggccttcgctcttcttagta

5'  aatggggaaggccatccagcctcgcgtcgcgaacgccagcaagacgtagcccagcgcgtcggccgccatg
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3430
3'  ttaccccttccggtaggtcggagcgcagcgcttgcggtcgttctgcatcgggtcgcgcagccggcggtac

5'  ccggcgataatggcctgcttctcgccgaaacgtttggtggcgggaccagtgacgaaggcttgagcgaggg
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3500
3'  ggccgctattaccggacgaagagcggctttgcaaaccaccgccctggtcactgcttccgaactcgctccc

5'  cgtgcaagattccgaataccgcaagcgacaggccgatcatcgtcgcgctccagcgaaagcggtcctcgcc
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3570
3'  gcacgttctaaggcttatggcgttcgctgtccggctagtagcagcgcgaggtcgctttcgccaggagcgg

5'  gaaaatgacccagagcgctgccggcacctgtcctacgagttgcatgataaagaagacagtcataagtgcg
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3640
3'  cttttactgggtctcgcgacggccgtggacaggatgctcaacgtactatttcttctgtcagtattcacgc

5'  gcgacgatagtcatgccccgcgcccaccggaaggagctgactgggttgaaggctctcaagggcatcggtc
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3710
3'  cgctgctatcagtacggggcgcgggtggccttcctcgactgacccaacttccgagagttcccgtagccag

5'  gagatcccggtgcctaatgagtgagctaacttacattaattgcgttgcgctcactgcccgctttccagtc
    ----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  3780
3'  ctctagggccacggattactcactcgattgaatgtaattaacgcaacgcgagtgacgggcgaaaggtcag
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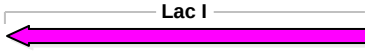
Lac I



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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
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4340
3'  tcttgaattacccgggcgattgtcgcgctaaacgaccactgggttacgctggtctacgaggtgcgggtca
                                   Lac I

5'  cgcgtaccgtcttcatgggagaaaataatactgttgatgggtgtctggtcagagacatcaagaaataacg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4410
3'  gcgcatggcagaagtaccctcttttattatgacaactacccacagaccagtctctgtagttctttattgc
                                   Lac I
```

```
5'  ccggaacattagtgcaggcagcttccacagcaatggcatcctggtcatccagcggatagttaatgatcag
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4480
3'  ggccttgtaatcacgtccgtcgaaggtgtcgttaccgtaggaccagtaggtcgcctatcaattactagtc
o                                   Lac I
o
o                MluI
5'  cccactgacgcgttgcgcgagaagattgtgcaccgccgctttacaggcttcgacgccgcttcgttctacc
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4550
3'  gggtgactgcgcaacgcgctcttctaacacgtggcggcgaaatgtccgaagctgcggcgaagcaagatgg
o                                   Lac I
o
5'  atcgacaccaccacgctggcacccagttgatcggcgcgagatttaatcgccgcgacaatttgcgacggcg
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4620
3'  tagctgtggtggtgcgaccgtgggtcaactagccgcgctctaaattagcggcgctgttaaacgctgccgc
o                                   Lac I
o
5'  cgtgcagggccagactggaggtggcaacgccaatcagcaacgactgtttgcccgccagttgttgtgccac
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4690
3'  gcacgtcccggtctgacctccaccgttgcggttagtcgttgctgacaaacgggcggtcaacaacacggtg
o                                   Lac I
o
5'  gcggttgggaatgtaattcagctccgccatcgccgcttccactttttcccgcgttttcgcagaaacgtgg
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4760
3'  cgccaacccttacattaagtcgaggcggtagcggcgaaggtgaaaaagggcgcaaaagcgtctttgcacc
o                   Lac I
o
5'  ctggcctggttcaccacgcgggaaacggtctgataagagacaccggcatactctgcgacatcgtataacg
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4830
3'  gaccggaccaagtggtgcgccctttgccagactattctctgtggccgtatgagacgctgtagcatattgc
o
5'  ttactggtttcacattcaccaccctgaattgactctcttccgggcgctatcatgccataccgcgaaaggt
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4900
3'  aatgaccaaagtgtaagtggtgggacttaactgagagaaggcccgcgatagtacggtatggcgctttcca
o
o                                                       EcoNI
5'  tttgcgccattcgatggtgtccgggatctcgacgctctcccttatgcgactcctgcattaggaagcagcc
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4970
3'  aaacgcggtaagctaccacaggccctagagctgcgagagggaatacgctgaggacgtaatccttcgtcgg
o
5'  cagtagtaggttgaggccgttgagcaccgccgccgcaaggaatggtgcatgcaaggagatggcgcccaac
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  5040
3'  gtcatcatccaactccggcaactcgtggcggcggcgttccttaccacgtacgttcctctaccgcgggttg
o
5'  agtcccccggccacggggcctgccaccatacccacgccgaaacaagcgctcatgagcccgaagtggcgag
o   ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  5110
3'  tcagggggccggtgccccggacggtggtatgggtgcggctttgttcgcgagtactcgggcttcaccgctc
o
```

SgrAI

5' cccgatcttccccatcggtgatgtcggcgatataggcgccagcaaccgcacctgtggcgccggtgatgcc

5180

3' gggctagaaggggtagccactacagccgctatatccgcggtcgttggcgtggacaccgcggccactacgg

5' ggccacgatgcgtccggcgtagaggatcgagatctcgatcccgcgaaattaatacgactcactatagggg

5250

3' ccggtgctacgcaggccgcatctcctagctctagagctagggcgctttaattatgctgagtgatatcccc

T7 Promoter

XbaI BarI NcoI

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' AACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttcaaggcACATCCTTGCCGAAGAACGGT

5460

3' TTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagttccgTGTAGGAACGGCTTCTTGCCA

attB1 TEV site BGH02_B02c

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

SgrDI

5' TTCAACATCGACCTTTCCGGTTCGTGGCAGCTCGCCTCCGTCGACGGCGAGATCCGCACCGCGATCATGC

5530

3' AAGTTGTAGCTGGAAAGGCCAAGCACCGTCGAGCGGAGGCAGCTGCCGCTCTAGGCGTGGCGCTAGTACG

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

5' TGCCGGGCGACGTGCACACGGCTCTGCACCGGGCAGGATTGATCCCCGATCCCTATTTCGGCCGCAACGA 5600
3' ACGGCCCGCTGCACGTGTGCCGAGACGTGGCCCGTCCTAACTAGGGGCTAGGGATAAAGCCGGCGTTGCT

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L P G D V H T A L H R A G L I P D P Y F G R N 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' GGAAAAGGTGCAGTGGGTCGCCGAGCGCGAATGGGCGGTCGAGCGCAGCTTCGCGCTTCAGGAGGTCGAG 5670
3' CCTTTTCCACGTCACCCAGCGGCTCGCGCTTACCCGCCAGCTCGCGTCGAAGCGCGAAGTCCTCCAGCTC

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E K V Q W V A E R E W A V E R S F A L Q E V E
96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118

5' GGCGACTGGTATCTCGATATCGATTATCTCGACACGGTTGCCAGCGTCCACGTCAACGGCTTTCTGGCGC 5740
3' CCGCTGACCATAGAGCTATAGCTAATAGAGCTGTGCCAACGGTCGCAGGTGCAGTTGCCGAAAGACCGCG

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G D W Y L D I D Y L D T V A S V H V N G F L A
119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141

5' TCGAAGCCGACAACAGCTTCCGCCGCTACCGGCCCGATGTCTCCAGCATGCTGAAATCAGGCGATAACAG 5810
3' AGCTTCGGCTGTTGTCGAAGGCGGCGATGGCCGGGCTACAGAGGTCGTACGACTTTAGTCCGCTATTGTC

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L E A D N S F R R Y R P D V S S M L K S G D N S
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' CATCCGCATCGTCTTTGCCTCCAACATCGCTGTCGGTGCCGCGCGACAGAAGCAGCAGCCCTTCTACATT 5880
3' GTAGGCGTAGCAGAAACGGAGGTTGTAGCGACAGCCACGGCGCGCTGTCTTCGTCGTCGGGAAGATGTAA

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

5' CCCTACAGCACGGGCAACTCACCGATCCCCGACGGCAACATGCTGCGCAAGCCGCAATGCCATTTCGGCT 5950
3' GGGATGTCGTGCCCGTTGAGTGGCTAGGGGCTGCCGTTGTACGACGCGTTCGGCGTTACGGTAAAGCCGA

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

5' GGGACTGGAACATCGCCATTGCCCCGCTCGGTCTTTACGGAACGATCGCGCTGAAAAAGCTCGAAACGGC 6020
3' CCCTGACCTTGTAGCGGTAACGGGGCGAGCCAGAAATGCCTTGCTAGCGCGACTTTTTCGAGCTTTGCCG

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W D W N I A I A P L G L Y G T I A L K K L E T A
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' GCGCATCGAACACGTCGTCACCCGCCAGACCCACAATAATGACGGTTCGGTCGACCTCAAAGTGACGGCG 6090
3' CGCGTAGCTTGTGCAGCAGTGGGCGGTCTGGGTGTTATTACTGCCAAGCCAGCTGGAGTTTCACTGCCGC

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

5' ACGCTGTTTTCCAAGGGGCCAGACATTGCCCAGGTCTATTTCGACCTCGATGGCGAGCGCGTGCGCCTGG 6160
3' TGCGACAAAAGGTTCCCCGGTCTGTAACGGGTCCAGATAAAGCTGGAGCTACCGCTCGCGCACGCGGACC

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

5' ATGTCGGCGTCAATGGCGAGACCCATGTCAACCATCTCTTCCATATCGACAATCCGCGCCTCTGGTGGCC 6230
3' TACAGCCGCAGTTACCGCTCTGGGTACAGTTGGTAGAGAAGGTATAGCTGTTAGGCGCGGAGACCACCGG

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D V G V N G E T H V N H L F H I D N P R L W W 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' CTCCGGCAGCGGCGAGCAGGCGCTCTACAGGCTTTCCGTCGAATTGCCGACGGATGAGGTGACCAAGCAG
6300
3' GAGGCCGTCGCCGCTCGTCCGCGAGATGTCCGAAAGGCAGCTTAACGGCTGCCTACTCCACTGGTTCGTC

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

5' ATCGGTCTTCGCACCATCGAGCTGATCACCACGCCGGATGCGGCCGGCAGCCGCTTTGCCTTCAAGGTCA
6370
3' TAGCCAGAAGCGTGGTAGCTCGACTAGTGGTGCGGCCTACGCCGGCCGTCGGCGAAACGGAAGTTCCAGT

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

5' ACGGCCGCGAGATCTTTTGCCGCGGCGCCAACTGGATCCCGGCCGACGCGCTGTTTTCGCTGTCTTCACC
6440
3' TGCCGGCGCTCTAGAAAACGGCGCCGCGGTTGACCTAGGGCCGGCTGCGCGACAAAAGCGACAGAAGTGG

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N G R E I F C R G A N W I P A D A L F S L S S P
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' TGAGAAGACCGAAGACCTGCTGCAATCGGCCAAAGCCGCCAACATGAACATGATCCGTGTCTGGGGCGGC
6510
3' ACTCTTCTGGCTTCTGGACGACGTTAGCCGGTTTCGGCGGTTGTACTTGTACTAGGCACAGACCCCGCCG

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

5' GGCTTCTATGAGCAGGATCATTTCTACGATCTCTGCGACCGGCTGGGCCTTATGGTCTGGCAGGACTTCA
6580
3' CCGAAGATACTCGTCCTAGTAAAGATGCTAGAGACGCTGGCCGACCCGGAATACCAGACCGTCCTGAAGT

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

5' TGTTTGCCTGCAACCTCTATCCGTCGACCGAGGACTTTCTCGACAATGTGACGATCGAGGTCGACTACCA 6650
3' ACAAACGGACGTTGGAGATAGGCAGCTGGCTCCTGAAAGAGCTGTTACACTGCTAGCTCCAGCTGATGGT

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M F A C N L Y P S T E D F L D N V T I E V D Y Q
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' GGTGCGCCGGCTCTCCTCGCATGCCTCGATCGTGCTCTGGTGCGGCGACAATGAACTGGTCGGAGCGCTC 6720
3' CCACGCGGCCGAGAGGAGCGTACGGAGCTAGCACGAGACCACGCCGCTGTTACTTGACCAGCCTCGCGAG

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V R R L S S H A S I V L W C G D N E L V G A L
446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468

5' ACCTGGTTCGAGGAATCGCGGAAGGATCGCGACCGCTATCTCGTCTCTTATGATCGCCTCAACCGCACGA 6790
3' TGGACCAAGCTCCTTAGCGCCTTCCTAGCGCTGGCGATAGAGCAGAGAATACTAGCGGAGTTGGCGTGCT

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

5' TCGAGCAGGCAGTGAAAAAGGCGCTGCCCGGCGCGGTCTGGTGGCCGTCGAGCCCGGCCTCCGGCTATCT 6860
3' AGCTCGTCCGTCACTTTTTCCGCGACGGGCCGCGCCAGACCACCGGCAGCTCGGGCCGGAGGCCGATAGA

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

NsiI

5' CGATTTCGGCGATGCCTGGCATGCGGACGGGTCCGGCGACATGCATTACTGGTCGGTCTGGCACGAGAAC 6930
3' GCTAAAGCCGCTACGGACCGTACGCCTGCCCAGGCCGCTGTACGTAATGACCAGCCAGACCGTGCTCTTG

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D F G D A W H A D G S G D M H Y W S V W H E N
516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538

5' AAGTCGTTCGACAATTACCGCTCGGTGCGTCCGCGCTTCTGCTCGGAATTCGGCTTCCAGTCCTATACGT 7000
3' TTCAGCAAGCTGTTAATGGCGAGCCACGCAGGCGCGAAGACGAGCCTTAAGCCGAAGGTCAGGATATGCA

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K S F D N Y R S V R P R F C S E F G F Q S Y T
539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561

5' CGCTGCCTGTGATCAAGACCTATGCCGAGGCGAAAGACATGAACGTCGCTTCCCCGGTCATGGAGCTGCA 7070
3' GCGACGGACACTAGTTCTGGATACGGCTCCGCTTTCTGTACTTGCAGCGAAGGGGCCAGTACCTCGACGT

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S L P V I K T Y A E A K D M N V A S P V M E L H
562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585

5' CCAGAAGAATGCCGGCGGCAACGAGCGCATCGCCGGCACGATGTTCCGTTATTTCCGATTCCCCAAGGAT 7140
3' GGTCTTCTTACGGCCGCCGTTGCTCGCGTAGCGGCCGTGCTACAAGGCAATAAAGGCTAAGGGGTTCCTA

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Q K N A G G N E R I A G T M F R Y F R F P K D
586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608

5' TTCCCGAACTTCGTCTATCTCTCCCAGATCCAACAGGGGCTGGCGATCAAGACGGCGGTGGAATACTGGC 7210
3' AAGGGCTTGAAGCAGATAGAGAGGGTCTAGGTTGTCCCCGACCGCTAGTTCTGCCGCCACCTTATGACCG

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F P N F V Y L S Q I Q Q G L A I K T A V E Y W
609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631

5' GGTCGCTCAAACCCCATTGCATGGGCACGATCTACTGGCAGCTCAACGACACCTGGCCGGTCGCCTCCTG 7280
3' CCAGCGAGTTTGGGGTAACGTACCCGTGCTAGATGACCGTCGAGTTGCTGTGGACCGGCCAGCGGAGGAC

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R S L K P H C M G T I Y W Q L N D T W P V A S W
632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655

NotI

5' GTCGAGCCTCGACTATGGCGGCCGCTGGAAGGCGATGCACTATCTCGTCAAGCGCTTCTTCCAGCCGGTC 7350
3' CAGCTCGGAGCTGATACCGCCGGCGACCTTCCGCTACGTGATAGAGCAGTTCGCGAAGAAGGTCGGCCAG

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S S L D Y G G R W K A M H Y L V K R F F Q P V
656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678

5' GCGGTCGCGGCCATTCCTTCCGACGACGGAAAGACGATTCGCTTCTCGCTGGTCAACGACACGCTTGCCG 7420
3' CGCCAGCGCCGGTAAGGAAGGCTGCTGCCTTTCTGCTAAGCGAAGAGCGACCAGTTGCTGTGCGAACGGC

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A V A A I P S D D G K T I R F S L V N D T L A
679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701

5' ATGTCAGCGTCGACCTTTCGATCTCGCTGCTGACGATGCAGGGCGAACGCCGGCACCTGAGAGACGTACA 7490
3' TACAGTCGCAGCTGGAAAGCTAGAGCGACGACTGCTACGTCCCGCTTGCGGCCGTGGACTCTCTGCATGT

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D V S V D L S I S L L T M Q G E R R H L R D V Q
702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725

5' GGCCATATGTTCGCCGGATGCGGCCGTCACTGCCACGAGCATCGACGTTTCCGATATTCCCGAGGGAACG 7560
3' CCGGTATACAAGCGGCCTACGCCGGCAGTGACGGTGCTCGTAGCTGCAAAGGCTATAAGGGCTCCCTTGC

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A I C S P D A A V T A T S I D V S D I P E G T
726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748

Acc65I
KpnI

5' TTGCTTGCCTGGCGTTTCACCGCGTCGAACGGCACGGGCGGCGAGGGGCACCATGTCAACGGTACCTACA 7630
3' AACGAACGGACCGCAAAGTGGCGCAGCTTGCCGTGCCCGCCGCTCCCCGTGGTACAGTTGCCATGGATGT

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L L A W R F T A S N G T G G E G H H V N G T Y
749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771

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5'  AGGCGCTGGAGCTGGAACCCGCGGGGCTGACAGTCACCCACGAATATGTCGAGGAGAATGGCTCGGTCGA
                                                                      7700
3'  TCCGCGACCTCGACCTTGGGCGCCCCGACTGTCAGTGGGTGCTTATACAGCTCCTCTTACCGAGCCAGCT
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    K   A   L   E   L   E   P   A   G   L   T   V   T   H   E   Y   V   E   E   N   G   S   V   D
    772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795

5'  CATCAACGTCACCGCGAAGGGACTTGCGCTTTTCGTGATGATCGAAACCGAGACCGACGGCAAATATTCC
                                                                      7770
3'  GTAGTTGCAGTGGCGCTTCCCTGAACGCGAAAAGCACTACTAGCTTTGGCTCTGGCTGCCGTTTATAAGG
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    I   N   V   T   A   K   G   L   A   L   F   V   M   I   E   T   E   T   D   G   K   Y   S
    796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818

5'  GACAACGCCTTCGATCTTGCGGCCGGCGAGAGCCGCCGCATCGCCTTTACCCCGGCACGGCCGCTCGATC
                                                                      7840
3'  CTGTTGCGGAAGCTAGAACGCCGGCCGCTCTCGGCGGCGTAGCGGAAATGGGGCCGTGCCGGCGAGCTAG
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    D   N   A   F   D   L   A   A   G   E   S   R   R   I   A   F   T   P   A   R   P   L   D
    819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841

5'  GTGGCGCGCTTCCGGAGTTCCGGTTCTACGACCTGTATTCCTGCCAGTCGGCGGATTAGGACCCAGCTTT
                                                                      7910
3'  CACCGCGCGAAGGCCTCAAGGCCAAGATGCTGGACATAAGGACGGTCAGCCGCCTAATCCTGGGTCGAAA
```

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    R   G   A   L   P   E   F   R   F   Y   D   L   Y   S   C   Q   S   A   D   .
    842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861

5'  CTTGTACAAAGTGGTTGATtcgaggatccggctgctaacaaagcccgaaaggaagctgagttggctgctg
                                                                      7980
3'  GAACATGTTTCACCAACTAagctcctaggccgacgattgtttcgggctttccttcgactcaaccgacgac
```

attB2

BlpI

```
5'  ccaccgctgagcaataactagcataaccccttggggcctctaaacgggtcttgaggggttttttgctgaa
                                                                      8050
3'  ggtggcgactcgttattgatcgtattggggaaccccggagatttgcccagaactccccaaaaaacgactt
```

T7 Terminator

```
5'  aggaggaactatatccggatatcccgcaagaggcccggcagtaccggcataaccaagcctatgcctacag
                                                                      8120
3'  tcctccttgatataggcctatagggcgttctccgggccgtcatggccgtattggttcggatacggatgtc
```

```
5'   catccagggtgacggtgccgaggatgacgatgagcgcattgttagatttcatacacggtgcctgactgcg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  8190
3'   gtaggtcccactgccacggctcctactgctactcgcgtaacaatctaaagtatgtgccacggactgacgc
o
                                        HindIII
o                                       |
5'   ttagcaatttaactgtgataaactaccgcattaaagcttatcgatgataagctgtcaaacatgagaa
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++  8257
3'   aatcgttaaattgacactatttgatggcgtaatttcgaatagctactattcgacagtttgtactctt
o
```