```
5'  tggcgaatgggacgcgccctgtagcggcgcattaagcgcggcgggtgtggtggttacgcgcagcgtgacc
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  70
3'  accgcttaccctgcgcgggacatcgccgcgtaattcgcgccgcccacaccaccaatgcgcgtcgcactgg

5'  gctacacttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  140
3'  cgatgtgaacggtcgcgggatcgcgggcgaggaaagcgaaagaagggaaggaaagagcggtgcaagcggc

5'  gctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagtgctttacggcacctcga
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  210
3'  cgaaaggggcagttcgagatttagcccccgagggaaatcccaaggctaaatcacgaaatgccgtggagct

                                          DraIII
                                          |
5'  ccccaaaaaacttgattagggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccct
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  280
3'  ggggttttttgaactaatcccactaccaagtgcatcacccggtagcgggactatctgccaaaaagcggga

     AlwI'                                AlwI
     |                                    |
5'  ttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacactcaaccctatct
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  350
3'  aactgcaacctcaggtgcaagaaattatcacctgagaacaaggtttgaccttgttgtgagttgggataga

                          PsiI
                          |
5'  cggtctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgattta
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  420
3'  gccagataagaaaactaaatattccctaaaacggctaaagccggataaccaatttttactcgactaaat

5'  acaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  490
3'  tgtttttaaattgcgcttaaaattgttttataattgcgaatgttaaatccaccgtgaaaagcccctttac

5'  tgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataacc
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  560
3'  acgcgccttggggataaacaaataaaaagatttatgtaagtttatacataggcgagtactctgttattgg

5'  ctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttatt
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  630
3'  gactatttacgaagttattataacttttccttctcatactcataagttgtaaaggcacagcgggaataa
                                                 [Ap]

5'  ccctttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  700
3'  gggaaaaaacgccgtaaaacggaaggacaaaaacgagtgggtctttgcgaccactttcattttctacgac
    [Ap]

5'  aagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttt
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  770
3'  ttctagtcaacccacgtgctcacccaatgtagcttgacctagagttgtcgccattctaggaactctcaaa
    [Ap]
```

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5'  tcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgt
                                                                      840
3'  agcggggcttcttgcaaaaggttactactcgtgaaaatttcaagacgatacaccgcgccataatagggca
                                  Ap

                                                          ScaI
5'  attgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcac
                                                                      910
3'  taactgcggcccgttctcgttgagccagcggcgtatgtgataagagtcttactgaaccaactcatgagtg
                                  Ap

5'  cagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgag
                                                                      980
3'  gtcagtgtcttttcgtagaatgcctaccgtactgtcattctcttaatacgtcacgacggtattggtactc
                                  Ap

5'  tgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgctttttgcac
                                                                     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
```

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5'  ggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaa
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1470
3'  cctacttgctttatctgtctagcgactctatccacggagtgactaattcgtaaccattgacagtctggtt
                                      Ap

5'  gtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcc
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1540
3'  caaatgagtatatatgaaatctaactaaattttgaagtaaaaattaaattttcctagatccacttctagg

5'  tttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtaga
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  1610
3'  aaaaactattagagtactggttttagggaattgcactcaaaagcaaggtgactcgcagtctggggcatct

5'  aaagatcaaaggatcttcttgagatccttttttctgcgcgtaatctgctgcttgcaaacaaaaaaacca
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  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
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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
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
```

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5'   tgggggtaatgataccgatgaaacgagagaggatgctcacgatacgggttactgatgatgaacatgcccg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2940
3'   acccccattactatggctactttgctctctcctacgagtgctatgcccaatgactactacttgtacgggc
o
5'   gttactggaacgttgtgagggtaaacaactggcggtatggatgcggcgggaccagagaaaaatcactcag
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3010
3'   caatgaccttgcaacactcccatttgttgaccgccatacctacgccgccctggtctctttttagtgagtc
o
5'   ggtcaatgccagcgcttcgttaatacagatgtaggtgttccacagggtagccagcagcatcctgcgatgc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3080
3'   ccagttacggtcgcgaagcaattatgtctacatccacaaggtgtcccatcggtcgtcgtaggacgctacg
o
5'   agatccggaacataatggtgcagggcgctgacttccgcgtttccagactttacgaaacacggaaaccgaa
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3150
3'   tctaggccttgtattaccacgtcccgcgactgaaggcgcaaaggtctgaaatgctttgtgcctttggctt
o
                         Bpu10I
o                        |
5'   gaccattcatgttgttgctcaggtcgcagacgttttgcagcagcagtcgcttcacgttcgctcgcgtatc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3220
3'   ctggtaagtacaacaacgagtccagcgtctgcaaaacgtcgtcgtcagcgaagtgcaagcgagcgcatag
o
5'   ggtgattcattctgctaaccagtaaggcaaccccgccagcctagccgggtcctcaacgacaggagcacga
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3290
3'   ccactaagtaagacgattggtcattccgttggggcggtcggatcggcccaggagttgctgtcctcgtgct
o
          FspAI
o         |
5'   tcatgcgcacccgtggggccgccatgccggcgataatggcctgcttctcgccgaaacgtttggtggcggg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3360
3'   agtacgcgtgggcaccccggcggtacggccgctattaccggacgaagagcggctttgcaaaccaccgccc
o
5'   accagtgacgaaggcttgagcgagggcgtgcaagattccgaataccgcaagcgacaggccgatcatcgtc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3430
3'   tggtcactgcttccgaactcgctcccgcacgttctaaggcttatggcgttcgctgtccggctagtagcag
o
5'   gcgctccagcgaaagcggtcctcgccgaaaatgacccagagcgctgccggcacctgtcctacgagttgca
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3500
3'   cgcgaggtcgctttcgccaggagcggcttttactgggtctcgcgacggccgtggacaggatgctcaacgt
o
                                         PshAI
o                                        |
5'   tgataaagaagacagtcataagtgcggcgacgatagtcatgccccgcgcccaccggaaggagctgactgg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3570
3'   actatttcttctgtcagtattcacgccgctgctatcagtacggggcgcgggtggccttcctcgactgacc
o
5'   gttgaaggctctcaagggcatcggtcgagatcccggtgcctaatgagtgagctaacttacattaattgcg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3640
3'   caacttccgagagttcccgtagccagctctagggccacggattactcactcgattgaatgtaattaacgc
o
5'   ttgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcggccaacgcg
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3710
3'   aacgcgagtgacgggcgaaaggtcagccctttggacagcacggtcgacgtaattacttagccggttgcgc
o
          Lac I
o         <------------------------------------------------------------------
```

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5'  cggggagaggcggtttgcgtattgggcgccagggtggtttttcttttcaccagtgagacgggcaacagct
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3780
3'  gcccctctccgccaaacgcataacccgcggtcccaccaaaaagaaaagtggtcactctgcccgttgtcga
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================

5'  gattgcccttcaccgcctggccctgagagagttgcagcaagcggtccacgctggtttgccccagcaggcg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3850
3'  ctaacgggaagtggcggaccgggactctctcaacgtcgttcgccaggtgcgaccaaacggggtcgtccgc
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================

                          HpaI
5'  aaaatcctgtttgatggtggttaacggcgggatataacatgagctgtcttcggtatcgtcgtatcccact
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3920
3'  ttttaggacaaactaccaccaattgccgccctatattgtactcgacagaagccatagcagcatagggtga
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================

5'  accgagatgtccgcaccaacgcgcagcccggactcggtaatggcgcgcattgcgcccagcgccatctgat
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3990
3'  tggctctacaggcgtggttgcgcgtcgggcctgagccattaccgcgcgtaacgcgggtcgcggtagacta
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================

5'  cgttggcaaccagcatcgcagtgggaacgatgccctcattcagcatttgcatggtttgttgaaaaccgga
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4060
3'  gcaaccgttggtcgtagcgtcacccttgctacgggagtaagtcgtaaacgtaccaaacaacttttggcct
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================

5'  catggcactccagtcgccttcccgttccgctatcggctgaatttgattgcgagtgagatatttatgccag
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4130
3'  gtaccgtgaggtcagcggaagggcaaggcgatagccgacttaaactaacgctcactctataaatacggtc
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================

                                          PspOMI
                                            ApaI                    BstEII
5'  ccagccagacgcagacgcgccgagacagaacttaatgggcccgctaacagcgcgatttgctggtgaccca
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4200
3'  ggtcggtctgcgtctgcgcggctctgtcttgaattacccgggcgattgtcgcgctaaacgaccactgggt
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================

5'  atgcgaccagatgctccacgcccagtcgcgtaccgtcttcatgggagaaaataatactgttgatgggtgt
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4270
3'  tacgctggtctacgaggtgcgggtcagcgcatggcagaagtaccctcttttattatgacaactacccaca
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================

5'  ctggtcagagacatcaagaaataacgccggaacattagtgcaggcagcttccacagcaatggcatcctgg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  4340
3'  gaccagtctctgtagttctttattgcggccttgtaatcacgtccgtcgaaggtgtcgttaccgtaggacc
    ------------------------------------------------------------------------
                                      Lac I
    ========================================================================
```

BclI MluI

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

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

RsrII

5' cggatgtactcaaagcggacggggcgatcctcgtcgatttctgggcagagtggtgcggtccgtgcaaaat 5320
3' gcctacatgagtttcgcctgccccgctaggagcagctaaagacccgtctcaccacgccaggcacgtttta

Trx Tag

5' gatcgccccgattctggatgaaatcgctgacgaatatcagggcaaactgaccgttgcaaaactgaacatc 5390
3' ctagcggggctaagacctactttagcgactgcttatagtcccgtttgactggcaacgttttgacttgtag

Trx Tag

5' gatcaaaaccctggcactgcgccgaaatatggcatccgtggtatcccgactctgctgctgttcaaaaacg 5460
3' ctagttttgggaccgtgacgcggctttataccgtaggcaccatagggctgagacgacgacaagtttttgc

Trx Tag

5' gtgaagtggcggcaaccaaagtgggtgcactgtctaaaggtcagttgaaagagttcctcgacgctaacct 5530
3' cacttcaccgccgttggtttcacccacgtgacagatttccagtcaactttctcaaggagctgcgattgga

Trx Tag

MscI

5' ggccggttctggttctggccatatgcaccatcatcatcatcattcttctggtctggtgccacgcggttct 5600
3' ccggccaagaccaagaccggtatacgtggtagtagtagtagtaagaagaccagaccacggtgcgccaaga

T...g — His6-tag — Thrombin

BstBI — BglII — Acc65I — KpnI

5' ggtatgaaagaaaccgctgctgctaaattcgaacgccagcacatggacagcccagatctgggtaccgacg 5670
3' ccatactttctttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagacccatggctgc

En...e

5' acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttca 5740
3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt

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5' aggcGCCTCACAGCTGAAACGGCGCACCACGATCGCGCGGCCAGTCCGCAAATCGCCGCCAGTATCGATC 5810
3' tccgCGGAGTGTCGACTTTGCCGCGTGGTGCTAGCGCGCCGGTCAGGCGTTTAGCGGCGGTCATAGCTAG

T... | BGT21_T01a | BGT21_T01a-pDONR221

A S Q L K R R T T I A R P V R K S P P V S I
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

AleI

5' GTCATACCGTCGCGCGGCGTCGAGCCGTTCACGCAGGAGACGCTGGAGCGCGCCTTCTCACTCGAGTGGC 5880
3' CAGTATGGCAGCGCGCCGCAGCTCGGCAAGTGCGTCCTCTGCGACCTCGCGCGGAAGAGTGAGCTCACCG

BGT21_T01a | BGT21_T01a-pDONR221

V I P S R G V E P F T Q E T L E R A F S L E W
23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

5' CGCGCTACGAACTGATCTTCTGCGTTGCCCATGGCGACGACCCGGTGGTCAGGTTGATCCGGGCCGCCAT 5950
3' GCGCGATGCTTGACTAGAAGACGCAACGGGTACCGCTGCTGGGCCACCAGTCCAACTAGGCCCGGCGGTA

BGT21_T01a | BGT21_T01a-pDONR221

P R Y E L I F C V A H G D D P V V R L I R A A I
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69

5' TGGCCGCTTTCCAAAAGTGCCGGCCCGGCTGTTGATCGGCGACGACCGCGTCAGCGCCAATCCCAAGCTC 6020
3' ACCGGCGAAAGGTTTTCACGGCCGGGCCGACAACTAGCCGCTGCTGGCGCAGTCGCGGTTAGGGTTCGAG

BGT21_T01a | BGT21_T01a-pDONR221

G R F P K V P A R L L I G D D R V S A N P K L
70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92

MfeI

5' AACAATTGCGTCAAGGGCTGGGAAGCCGCGCGGCACAACTGGGTCGTCCTGGCCGACTCCAACGTGCTGA 6090
3' TTGTTAACGCAGTTCCCGACCCTTCGGCGCGCCGTGTTGACCCAGCAGGACCGGCTGAGGTTGCACGACT

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BGT21_T01a-pDONR221

N N C V K G W E A A R H N W V V L A D S N V L
93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115

5' TGCCGAGAGACTACATCCAGCATCTGATGGCGGCGTGGCGGCCGGATACCGGCCTTGTCTGCTCGACGCC 6160
3' ACGGCTCTCTGATGTAGGTCGTAGACTACCGCCGCACCGCCGGCCTATGGCCGGAACAGACGAGCTGCGG

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BGT21_T01a-pDONR221

M P R D Y I Q H L M A A W R P D T G L V C S T P
116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139

5' GATCGGCTCGCGGCCGGAGGGCTTCTGGGCCGAGGTCGAATGCGCCTTCCTCAACACGCTGCAGGCCCGC 6230
3' CTAGCCGAGCGCCGGCCTCCCGAAGACCCGGCTCCAGCTTACGCGGAAGGAGTTGTGCGACGTCCGGGCG

BGT21_T01a

BGT21_T01a-pDONR221

I G S R P E G F W A E V E C A F L N T L Q A R
140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162

PasI

5' TGGCAGTATGCTGGCGAGGCGCTCGGCCTTGGCTTTGCCCAGGGCAAGAGCATGCTGTGGAACAAGCCGA 6300
3' ACCGTCATACGACCGCTCCGCGAGCCGGAACCGAAACGGGTCCCGTTCTCGTACGACACCTTGTTCGGCT

BGT21_T01a

BGT21_T01a-pDONR221

W Q Y A G E A L G L G F A Q G K S M L W N K P
163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185

5' TGCTGGATGCCAATGGCGGCATCCGCGCGCTGGCCGCTGAAATCGCCGAGGACGCCGCCGCAACCAAGCT 6370
3' ACGACCTACGGTTACCGCCGTAGGCGCGCGACCGGCGACTTTAGCGGCTCCTGCGGCGGCGTTGGTTCGA

BGT21_T01a

BGT21_T01a-pDONR221

M L D A N G G I R A L A A E I A E D A A A T K L
186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209

```
5'  GGTCAATGGGCTTGGCCTGCGAGTCAATCTTGTCGCGGCACCCTTCGAGCAGCCGCTTGGCCGGCGCACG
                                                                          6440
3'  CCAGTTACCCGAACCGGACGCTCAGTTAGAACAGCGCCGTGGGAAGCTCGTCGGCGAACCGGCCGCGTGC
```

BGT21_T01a

BGT21_T01a-pDONR221

```
V  N  G  L  G  L  R  V  N  L  V  A  A  P  F  E  Q  P  L  G  R  R  T
210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232
```

AscI

```
5'  CTGGACGCGATCTGGTCGCGCCAGGCTCGCTGGGCGCGCCTGCGCCGCGTCACCTTCCCGCTGTTCTTCG
                                                                          6510
3'  GACCTGCGCTAGACCAGCGCGGTCCGAGCGACCCGCGCGGACGCGGCGCAGTGGAAGGGCGACAAGAAGC
```

BGT21_T01a

BGT21_T01a-pDONR221

```
L  D  A  I  W  S  R  Q  A  R  W  A  R  L  R  R  V  T  F  P  L  F  F
233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255

5'  CGCCGGAAATCCTGACTGGCGCGGTGGTGCCGTTGATGCTTGCACTGGTTGCGGCGGCAAGCGCCGGCTT
                                                                          6580
3'  GCGGCCTTTAGGACTGACCGCGCCACCACGGCAACTACGAACGTGACCAACGCCGCCGTTCGCGGCCGAA
```

BGT21_T01a

BGT21_T01a-pDONR221

```
A  P  E  I  L  T  G  A  V  V  P  L  M  L  A  L  V  A  A  A  S  A  G  F
256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279

5'  CAGCCTGCCGCTGACGGCCATCGCCGTGCTGGCCGCCTTCTACCTTCCGGAATGCGCCCTGGCATGGTCC
                                                                          6650
3'  GTCGGACGGCGACTGCCGGTAGCGGCACGACCGGCGGAAGATGGAAGGCCTTACGCGGGACCGTACCAGG
```

BGT21_T01a

BGT21_T01a-pDONR221

```
S  L  P  L  T  A  I  A  V  L  A  A  F  Y  L  P  E  C  A  L  A  W  S
280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302

5'  AAGGGCTGGCACCTGTCGCCGCGCACGGTCGCGGCGATGATTGCGCGGGACTTGATGGTCCCAGCTATGT
                                                                          6720
3'  TTCCCGACCGTGGACAGCGGCGCGTGCCAGCGCCGCTACTAACGCGCCCTGAACTACCAGGGTCGATACA
```

BGT21_T01a

BGT21_T01a-pDONR221

```
K  G  W  H  L  S  P  R  T  V  A  A  M  I  A  R  D  L  M  V  P  A  M
303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325
```

```
5'  GGGCACGCGGCTGGCTTGGCGGCGCCGTCGACTGGCGCGGCAATGCGATGACCATCGGCACCAAGGCGGC
                                                                          6790
3'  CCCGTGCGCCGACCGAACCGCCGCGGCAGCTGACCGCGCCGTTACGCTACTGGTAGCCGTGGTTCCGCCG
    ------------------------------------- BGT21_T01a -------------------------------------
    ------------------------------------- BGT21_T01a-pDONR221 ---------------------------------

    W   A   R   G   W   L   G   G   A   V   D   W   R   G   N   A   M   T   I   G   T   K   A   A
    326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349

                                        AvrII                                       BamHI
5'  CGAACTGGAAGAGGCGTCGTCGCGCGCCTAGGACCCAGCTTTCTTGTACAAAGTGGTTGATatcggatcc
                                                                          6860
3'  GCTTGACCTTCTCCGCAGCAGCGCGCGGATCCTGGGTCGAAAGAACATGTTTCACCAACTAtagcctagg
    ---------- BGT21_T01a ----------          ---------- attB2 ----------
    ---------- BGT21_T01a-pDONR221 ----------

    E   L   E   E   A   S   S   R   A   .
    350 351 352 353 354 355 356 357 358 359

                EcolCRI
    EcoRI       SacI        HindIII     NotI
5'  gaattcgagctccgtcgacaagcttgcggccgcactcgagcaccaccaccaccaccactgagatccggct
                                                                          6930
3'  cttaagctcgaggcagctgttcgaacgccggcgtgagctcgtggtggtggtggtggtgactctaggccga

                                                    BlpI
5'  gctaacaaagcccgaaaggaagctgagttggctgctgccaccgctgagcaataactagcataaccccttg
                                                                          7000
3'  cgattgtttcgggctttccttcgactcaaccgacgacggtggcgactcgttattgatcgtattggggaac
                                                  ---------- T7 Terminator ----------

5'  gggcctctaaacgggtcttgaggggttttttgctgaaaggaggaactatatccggat
                                                                          7057
3'  cccggagatttgcccagaactccccaaaaaacgactttcctccttgatataggccta
    ---------- T7 Terminator ----------
```