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

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'  aggaccgaaggagctaaccgctttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaa
    ----|----|----|----|----|----|----|----|----|----|----|----|----|----|  700
3'  tcctggcttcctcgattggcgaaaaaacgtgttgtacccccctagtacattgagcggaactagcaaccctt
    Ap
```

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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
```

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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
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
```

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

                        AccI
o                       BstZ17I                     BsaAI    Tth111I

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
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                                                                                  AvaI
                                                FspAI                             BmeT110I
5'  cctagccgggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgcccgaga
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  2940
3'  ggatcggcccaggagttgctgtcctcgtgctagtacgcgtgggcaccggtcctgggttgcgacgggctct

                                                                      BsmI
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

                                                                  EagI
5'  aatggggaaggccatccagcctcgcgtcgcgaacgccagcaagacgtagcccagcgcgtcggccgccatg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3430
3'  ttaccccttccggtaggtcggagcgcagcgcttgcggtcgttctgcatcgggtcgcgcagccggcggtac

5'  ccggcgataatggcctgcttctcgccgaaacgtttggtggcgggaccagtgacgaaggcttgagcgaggg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3500
3'  ggccgctattaccggacgaagagcggctttgcaaaccaccgccctggtcactgcttccgaactcgctccc

5'  cgtgcaagattccgaataccgcaagcgacaggccgatcatcgtcgcgctccagcgaaagcggtcctcgcc
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3570
3'  gcacgttctaaggcttatggcgttcgctgtccggctagtagcagcgcgaggtcgctttcgccaggagcgg

5'  gaaaatgacccagagcgctgccggcacctgtcctacgagttgcatgataaagaagacagtcataagtgcg
    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  3640
3'  cttttactgggtctcgcgacggccgtggacaggatgctcaacgtactatttcttctgtcagtattcacgc
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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

5' gagagttgcagcaagcggtccacgctggtttgccccagcaggcgaaaatcctgtttgatggtggttaacg 3990
3' ctctcaacgtcgttcgccaggtgcgaccaaacggggtcgtccgcttttaggacaaactaccaccaattgc

Lac I

5' gcgggatataacatgagctgtcttcggtatcgtcgtatcccactaccgagatatccgcaccaacgcgcag 4060
3' cgccctatattgtactcgacagaagccatagcagcatagggtgatggctctataggcgtggttgcgcgtc

Lac I

BssHII

5' cccggactcggtaatggcgcgcattgcgcccagcgccatctgatcgttggcaaccagcatcgcagtggga 4130
3' gggcctgagccattaccgcgcgtaacgcgggtcgcggtagactagcaaccgttggtcgtagcgtcaccct

Lac I

5' acgatgccctcattcagcatttgcatggtttgttgaaaaccggacatggcactccagtcgccttcccgtt 4200
3' tgctacgggagtaagtcgtaaacgtaccaaacaacttttggcctgtaccgtgaggtcagcggaagggcaa

Lac I

5' ccgctatcggctgaatttgattgcgagtgagatatttatgccagccagccagacgcagacgcgccgagac 4270
3' ggcgatagccgacttaaactaacgctcactctataaatacggtcggtcggtctgcgtctgcgcggctctg

Lac I

PspOMI
ApaI
BstEII

5' agaacttaatgggcccgctaacagcgcgatttgctggtgacccaatgcgaccagatgctccacgcccagt 4340
3' tcttgaattacccgggcgattgtcgcgctaaacgaccactgggttacgctggtctacgaggtgcgggtca
Lac I

5' cgcgtaccgtcttcatgggagaaaataatactgttgatgggtgtctggtcagagacatcaagaaataacg 4410
3' gcgcatggcagaagtaccctcttttattatgacaactacccacagaccagtctctgtagttctttattgc
Lac I

5' ccggaacattagtgcaggcagcttccacagcaatggcatcctggtcatccagcggatagttaatgatcag 4480
3' ggccttgtaatcacgtccgtcgaaggtgtcgttaccgtaggaccagtaggtcgcctatcaattactagtc
Lac I

MluI

5' cccactgacgcgttgcgcgagaagattgtgcaccgccgctttacaggcttcgacgccgcttcgttctacc 4550
3' gggtgactgcgcaacgcgctcttctaacacgtggcggcgaaatgtccgaagctgcggcgaagcaagatgg
Lac I

5' atcgacaccaccacgctggcacccagttgatcggcgcgagatttaatcgccgcgacaatttgcgacggcg 4620
3' tagctgtggtggtgcgaccgtgggtcaactagccgcgctctaaattagcggcgctgttaaacgctgccgc
Lac I

5' cgtgcagggccagactggaggtggcaacgccaatcagcaacgactgtttgcccgccagttgttgtgccac 4690
3' gcacgtcccggtctgacctccaccgttgcggttagtcgttgctgacaaacgggcggtcaacaacacggtg
Lac I

5' gcggttgggaatgtaattcagctccgccatcgccgcttccactttttcccgcgttttcgcagaaacgtgg 4760
3' cgccaacccttacattaagtcgaggcggtagcggcgaaggtgaaaaagggcgcaaaagcgtctttgcacc
Lac I

5' ctggcctggttcaccacgcgggaaacggtctgataagagacaccggcatactctgcgacatcgtataacg 4830
3' gaccggaccaagtggtgcgccctttgccagactattctctgtggccgtatgagacgctgtagcatattgc

5' ttactggtttcacattcaccaccctgaattgactctcttccgggcgctatcatgccataccgcgaaaggt 4900
3' aatgaccaaagtgtaagtggtgggacttaactgagagaaggcccgcgatagtacggtatggcgctttcca

EcoNI

5' tttgcgccattcgatggtgtccgggatctcgacgctctcccttatgcgactcctgcattaggaagcagcc 4970
3' aaacgcggtaagctaccacaggccctagagctgcgagagggaatacgctgaggacgtaatccttcgtcgg

5' cagtagtaggttgaggccgttgagcaccgccgccgcaaggaatggtgcatgcaaggagatggcgcccaac 5040
3' gtcatcatccaactccggcaactcgtggcggcggcgttccttaccacgtacgttcctctaccgcgggttg

5' agtcccccggccacggggcctgccaccatacccacgccgaaacaagcgctcatgagcccgaagtggcgag 5110
3' tcagggggccggtgccccggacggtggtatgggtgcggctttgttcgcgagtactcgggcttcaccgctc

SgrAI

5' cccgatcttccccatcggtgatgtcggcgatataggcgccagcaaccgcacctgtggcgccggtgatgcc 5180
3' gggctagaaggggtagccactacagccgctatatccgcggtcgttggcgtggacaccgcggccactacgg

5' ggccacgatgcgtccggcgtagaggatcgagatctcgatcccgcgaaattaatacgactcactatagggg 5250
3' ccggtgctacgcaggccgcatctcctagctctagagctagggcgctttaattatgctgagtgatatcccc

T7 Promoter

XbaI NcoI

5' aattgtgagcggataacaattcccctctagaaataattttgtttaactttaagaaggagatataccatgg 5320
3' ttaacactcgcctattgttaaggggagatctttattaaaacaaattgaaattcttcctctatatggtacc

Lac O

M
1

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' AACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttcaaggcTCACAACCTATTTTTAACGAT 5460
3' TTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagttccgAGTGTTGGATAAAAATTGCTA

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

AleI BseRI

5' AAGCAATTTCAGGAAGCGCTTTCACGTCAGTGGCAGCGTTATGGCTTAAATTCTGCGGCTGAAATGACTC 5530
3' TTCGTTAAAGTCCTTCGCGAAAGTGCAGTCACCGTCGCAATACCGAATTTAAGACGCCGACTTTACTGAG

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K Q F Q E A L S R Q W Q R Y G L N S A A E M 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

5' CTCGCCAGTGGTGGCTAGCAGTGAGTGAAGCACTGGCCGAAATGCTGCGTGCTCAGCCATTCGCCAAGCC 5600
3' GAGCGGTCACCACCGATCGTCACTCACTTCGTGACCGGCTTTACGACGCACGAGTCGGTAAGCGGTTCGG

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P R Q W W L A V S E A L A E M L R A Q P F A K P
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' GGTGGCGAATCAGCGACATGTTAACTACATCTCAATGGAGTTTTTGATTGGTCGCCTGACGGGCAACAAC 5670
3' CCACCGCTTAGTCGCTGTACAATTGATGTAGAGTTACCTCAAAAACTAACCAGCGGACTGCCCGTTGTTG

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

5' CTGTTGAATCTCGGCTGGTATCAGGATGTACAGGATTCGTTGAAGGCTTATGACATCAATCTGACGGACC 5740
3' GACAACTTAGAGCCGACCATAGTCCTACATGTCCTAAGCAACTTCCGAATACTGTAGTTAGACTGCCTGG

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

5' TGCTGGAAGAAGAGATCGACCCGGCGCTGGGTAACGGTGGTCTGGGACGTCTGGCGGCGTGCTTCCTCGA 5810
3' ACGACCTTCTTCTCTAGCTGGGCCGCGACCCATTGCCACCAGACCCTGCAGACCGCCGCACGAAGGAGCT

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L L E E E I D P A L G N G G L G R L A A C F L 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

5' CTCAATGGCAACTGTCGGTCAGTCTGCGACGGGTTACGGTCTGAACTATCAATATGGTTTGTTCCGCCAG 5880
3' GAGTTACCGTTGACAGCCAGTCAGACGCTGCCCAATGCCAGACTTGATAGTTATACCAAACAAGGCGGTC

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

5' TCTTTTGTCGATGGCAAACAGGTTGAAGCGCCGGATGACTGGCATCGCAGTAACTACCCGTGGTTCCGCC 5950
3' AGAAAACAGCTACCGTTTGTCCAACTTCGCGGCCTACTGACCGTAGCGTCATTGATGGGCACCAAGGCGG

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

5' ACAACGAAGCACTGGATGTGCAGGTAGGGATTGGCGGTAAAGTGACGAAAGACGGACGCTGGGAGCCGGA 6020
3' TGTTGCTTCGTGACCTACACGTCCATCCCTAACCGCCATTTCACTGCTTTCTGCCTGCGACCCTCGGCCT

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H N E A L D V Q V G I G G K V T K D G R W E P E
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' GTTTACCATTACCGGTCAAGCGTGGGATCTCCCCGTTGTCGGCTATCGTAATGGCGTGGCGCAGCCGCTG 6090
3' CAAATGGTAATGGCCAGTTCGCACCCTAGAGGGGCAACAGCCGATAGCATTACCGCACCGCGTCGGCGAC

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

5' CGTCTGTGGCAGGCGACGCACGCGCATCCGTTTGATCTGACTAAATTTAACGACGGTGATTTCTTGCGTG 6160
3' GCAGACACCGTCCGCTGCGTGCGCGTAGGCAAACTAGACTGATTTAAATTGCTGCCACTAAAGAACGCAC

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

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5'  CCGAACAGCAGGGCATCAATGCGGAAAAACTGACCAAAGTTCTCTATCCAAACGACAACCATACTGCCGG
                                                                      6230
3'  GGCTTGTCGTCCCGTAGTTACGCCTTTTTGACTGGTTTCAAGAGATAGGTTTGCTGTTGGTATGACGGCC
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A  E  Q  Q  G  I  N  A  E  K  L  T  K  V  L  Y  P  N  D  N  H  T  A  G
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'  TAAAAAGCTGCGCCTGATGCAGCAATACTTCCAGTGTGCCTGTTCGGTAGCGGATATTTTGCGTCGCCAT
                                                                      6300
3'  ATTTTTCGACGCGGACTACGTCGTTATGAAGGTCACACGGACAAGCCATCGCCTATAAAACGCAGCGGTA
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K  K  L  R  L  M  Q  Q  Y  F  Q  C  A  C  S  V  A  D  I  L  R  R  H
306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328

5'  CATCTGGCGGGGCGTAAACTGCACGAACTGGCGGATTACGAAGTTATTCAGCTGAACGATACCCACCCAA
                                                                      6370
3'  GTAGACCGCCCCGCATTTGACGTGCTTGACCGCCTAATGCTTCAATAAGTCGACTTGCTATGGGTGGGTT
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H  L  A  G  R  K  L  H  E  L  A  D  Y  E  V  I  Q  L  N  D  T  H  P
329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351

5'  CTATCGCGATTCCAGAACTGCTGCGCGTGCTGATCGATGAGCACCAGATGAGCTGGGATGACGCCTGGGC
                                                                      6440
3'  GATAGCGCTAAGGTCTTGACGACGCGCACGACTAGCTACTCGTGGTCTACTCGACCCTACTGCGGACCCG
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T  I  A  I  P  E  L  L  R  V  L  I  D  E  H  Q  M  S  W  D  D  A  W  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

5'  CATTACCAGCAAAACTTTCGCTTACACCAACCATACCCTGATGCCAGAAGCGCTGGAACGCTGGGATGTG
                                                                      6510
3'  GTAATGGTCGTTTTGAAAGCGAATGTGGTTGGTATGGGACTACGGTCTTCGCGACCTTGCGACCCTACAC
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I  T  S  K  T  F  A  Y  T  N  H  T  L  M  P  E  A  L  E  R  W  D  V
376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398
```

5' AAACTGGTGAAAGGCTTACTGCCGCGCCACATGCAGATTATTAACGAAATTAATACTCGCTTTAAAACGC 6580
3' TTTGACCACTTTCCGAATGACGGCGCGGTGTACGTCTAATAATTGCTTTAATTATGAGCGAAATTTTGCG

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K L V K G L L P R H M Q I I N E I N T R F K T
399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421

5' TGGTAGAGAAAACCTGGCCGGGCGATGAAAAAGTGTGGGCCAAACTGGCGGTGGTGCACGACAAACAAGT 6650
3' ACCATCTCTTTTGGACCGGCCCGCTACTTTTTCACACCCGGTTTGACCGCCACCACGTGCTGTTTGTTCA

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L V E K T W P G D E K V W A K L A V V H D K Q V
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' GCATATGGCGAACCTGTGTGTGGTTGGCGGTTTCGCGGTGAACGGTGTTGCGGCGCTGCACTCGGATCTG 6720
3' CGTATACCGCTTGGACACACACCAACCGCCAAAGCGCCACTTGCCACAACGCCGCGACGTGAGCCTAGAC

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H M A N L C V V G G F A V N G V A A L H S D 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' GTGGTGAAAGATCTGTTCCCGGAATATCACCAGCTATGGCCGAACAAATTCCATAACGTCACCAACGGTA 6790
3' CACCACTTTCTAGACAAGGGCCTTATAGTGGTCGATACCGGCTTGTTTAAGGTATTGCAGTGGTTGCCAT

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

CspCI

5' TTACCCCACGTCGCTGGATCAAACAGTGCAACCCGGCACTGGCGGCTCTGTTGGATAAATCACTGCAAAA 6860
3' AATGGGGTGCAGCGACCTAGTTTGTCACGTTGGGCCGTGACCGCCGAGACAACCTATTTAGTGACGTTTT

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

CspCI'

5' AGAGTGGGCTAACGATCTCGATCAGCTGATCAATCTGGAAAAATTCGCTGATGATGCGAAATTCCGTCAG 6930
3' TCTCACCCGATTGCTAGAGCTAGTCGACTAGTTAGACCTTTTTAAGCGACTACTACGCTTTAAGGCAGTC

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E W A N D L D Q L I N L E K F A D D A K F R Q
516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538

5' CAATATCGCGAGATCAAGCAGGCGAATAAAGTCCGTCTGGCGGAGTTTGTGAAAGTTCGTACCGGTATTG 7000
3' GTTATAGCGCTCTAGTTCGTCCGCTTATTTCAGGCAGACCGCCTCAAACACTTTCAAGCATGGCCATAAC

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

5' AGATCAATCCACAGGCGATTTTCGATATTCAGATCAAACGTTTGCATGAGTACAAACGCCAGCACCTGAA 7070
3' TCTAGTTAGGTGTCCGCTAAAAGCTATAAGTCTAGTTTGCAAACGTACTCATGTTTGCGGTCGTGGACTT

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E I N P Q A I F D I Q I K R L H E Y K R Q H L N
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' TCTGCTGCATATTCTGGCGTTGTACAAAGAAATTCGTGAAAACCCGCAGGCTGATCGCGTACCGCGCGTC 7140
3' AGACGACGTATAAGACCGCAACATGTTTCTTTAAGCACTTTTGGGCGTCCGACTAGCGCATGGCGCGCAG

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

5' TTCCTCTTCGGCGCGAAAGCGGCACCGGGCTACTACCTGGCGAAGAATATTATCTTTGCGATCAACAAAG 7210
3' AAGGAGAAGCCGCGCTTTCGCCGTGGCCCGATGATGGACCGCTTCTTATAATAGAAACGCTAGTTGTTTC

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

5' TGGCTGACGTGATCAACAACGATCCGCTGGTTGGCGATAAGTTGAAGGTGGTGTTCCTGCCGGATTATTG 7280
3' ACCGACTGCACTAGTTGTTGCTAGGCGACCAACCGCTATTCAACTTCCACCACAAGGACGGCCTAATAAC

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

5' CGTTTCGGCGGCGGAAAAACTGATCCCGGCGGCGGATATCTCCGAACAAATTTCGACTGCAGGTAAAGAA 7350
3' GCAAAGCCGCCGCCTTTTTGACTAGGGCCGCCGCCTATAGAGGCTTGTTTAAAGCTGACGTCCATTTCTT

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V S A A E K L I P A A D I S E Q I S T A G K E
656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678

Acc65I
KpnI

5' GCTTCCGGTACCGGCAATATGAAACTGGCGCTCAATGGTGCGCTTACTGTCGGTACGCTGGATGGGGCGA 7420
3' CGAAGGCCATGGCCGTTATACTTTGACCGCGAGTTACCACGCGAATGACAGCCATGCGACCTACCCCGCT

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A S G T G N M K L A L N G A L T V G T L D G 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' ACGTTGAAATCGCCGAGAAAGTCGGTGAAGAAAATATCTTTATTTTTGGTCATACCGTGGAACAAGTGAA 7490
3' TGCAACTTTAGCGGCTCTTTCAGCCACTTCTTTTATAGAAATAAAAACCAGTATGGCACCTTGTTCACTT

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N V E I A E K V G E E N I F I F G H T V E Q V K
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' GGCAATTCTGGCCAAAGGCTACGACCCGGTGAAATGGCGGAAGAAAGATAAGGTGCTGGACGCAGTATTG 7560
3' CCGTTAAGACCGGTTTCCGATGCTGGGCCACTTTACCGCCTTCTTTCTATTCCACGACCTGCGTCATAAC

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A I L A K G Y D P V K W R K K D K V L D A V L
726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748

5' AAAGAGCTGGAAAGCGGTAAATACAGCGACGGCGATAAGCATGCCTTCGACCAGATGCTGCACAGTATCG 7630
3' TTTCTCGACCTTTCGCCATTTATGTCGCTGCCGCTATTCGTACGGAAGCTGGTCTACGACGTGTCATAGC

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K E L E S G K Y S D G D K H A F D Q M L H S I
749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771

AarI

5' GCAAACAGGGCGGCGATCCGTATCTGGTGATGGCGGATTTCGCAGCCTATGTAGAGGCACAAAAGCAGGT 7700
3' CGTTTGTCCCGCCGCTAGGCATAGACCACTACCGCCTAAAGCGTCGGATACATCTCCGTGTTTTCGTCCA

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G K Q G G D P Y L V M A D F A A Y V E A Q K Q V
772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795

StuI

5' GGATGTGCTGTACCGCGACCAGGAGGCCTGGACTCGCGCGGCGATCCTCAATACCGCCCGCTGCGGTATG 7770
3' CCTACACGACATGGCGCTGGTCCTCCGGACCTGAGCGCGCCGCTAGGAGTTATGGCGGGCGACGCCATAC

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D V L Y R D Q E A W T R A A I L N T A R C G M
796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818

5' TTTAGCTCGGATCGCTCTATTCGCGATTATCAGGCTCGTATCTGGCAGGCAAAACGCTAGGACCCAGCTT 7840
3' AAATCGAGCCTAGCGAGATAAGCGCTAATAGTCCGAGCATAGACCGTCCGTTTTGCGATCCTGGGTCGAA

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attB2

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F S S D R S I R D Y Q A R I W Q A K R .
819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838

BamHI

5' TCTTGTACAAAGTGGTTGATtcgaggatccggctgctaacaaagcccgaaaggaagctgagttggctgct 7910
3' AGAACATGTTTCACCAACTAagctcctaggccgacgattgtttcgggctttccttcgactcaaccgacga

attB2

5' gccaccgctgagcaataactagcataaccccttggggcctctaaacgggtcttgaggggttttttgctga 7980
3' cggtggcgactcgttattgatcgtattggggaaccccggagatttgcccagaactccccaaaaaacgact

T7 Terminator

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5'   aaggaggaactatatccggatatcccgcaagaggcccggcagtaccggcataaccaagcctatgcctaca
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  8050
3'   ttcctccttgatataggcctatagggcgttctccgggccgtcatggccgtattggttcggatacggatgt
o

5'   gcatccagggtgacggtgccgaggatgacgatgagcgcattgttagatttcatacacggtgcctgactgc
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  8120
3'   cgtaggtcccactgccacggctcctactgctactcgcgtaacaatctaaagtatgtgccacggactgacg
o

                                                                    EcoRI
o                                                                     |
5'   gttagcaatttaactgtgataaactaccgcattaaagcttatcgatgataagctgtcaaacatgagaa
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++   8188
3'   caatcgttaaattgacactatttgatggcgtaatttcgaatagctactattcgacagtttgtactctt
o
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