pmKate2-endo vector restriction map

The data has not been verified by restriction digestion with each enzyme listed and does not take into account possible methylation sites. Enzymes that recognize unambiguous sequences less than 6 basepairs long are not included – for the more complete enzyme list please refer to the Table of restriction sites.

Unique sites are shown in bold blue. The location given specifies the 3' end of the cut DNA (the base to the left of the cut site). mKate2 amino acids are shown in red, c-Myc epitope and Human RhoB GTPase amino acids are shown in green, linker amino acids are shown in black.

```
        Ase1                                                                               Bgl1
           |                                                                                  |
     TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCG
  1  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 100
     ATCAATAATTATCATTAGTTAATGCCCCAGTAATCAAGTATCGGGTATATACCTCAAGGCGCAATGTATTGAATGCCATTTACCGGGCGGACCGACTGGC

                          Aat2                   SpAcc                         Aat2
                             |                       |                            |
     CCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT
101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 200
     GGGTTGCTGGGGGCGGGTAACTGCAGTTATTACTGCATACAAGGGTATCATTGCGGTTATCCCTGAAAGGTAACTGCAGTTACCCACCTCATAAATGCCA

             Bgl1                  Nde1                       Aat2                  Bgl1  Bmr1
                |                     |                          |                     |     |
     AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTA
201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 300
     TTTGACGGGTGAACCGTCATGTAGTTCACATAGTATACGGTTCATGCGGGGGATAACTGCAGTTACTGCCATTTACCGGGCGGACCGTAATACGGGTCAT

                                  BtgZ1 SnaB1                Nco1
                                      |     |                   |
     CATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGA
301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 400
     GTACTGGAATACCCTGAAAGGATGAACCGTCATGTAGATGCATAATCAGTAGCGATAATGGTACCACTACGCCAAAACCGTCATGTAGTTACCCGCACCT
```

```
                                                          Aat2                                             Eci1
                                                             |                                                |
               TAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA
          401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 500
               ATCGCCAAACTGAGTGCCCCTAAAGGTTCAGAGGTGGGGTAACTGCAGTTACCCTCAAACAAAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCAT

                                                                                                      Nhe1 Afe1
                                                                                                         |    |
               ACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTA
          501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 600
               TGTTGAGGCGGGGTAACTGCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGACCAAATCACTTGGCAGTCTAGGCGATCGCGAT

                         SpDon
                        Ale1 |
           Age1       Nco1 | |                                 BsrG1                                ApaL1
              |          | | |                                     |                                    |
               CCGGTAGCCACCATGGTGAGCGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCG
          601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 700
               GGCCATCGGTGGTACCACTCGCTCGACTAATTCCTCTTGTACGTGTACTTCGACATGTACCTCCCGTGGCACTTGTTGGTGGTGAAGTTCACGTGTAGGC
mKate2       >             M  V  S  E  L  I  K  E  N  M  H  M  K  L  Y  M  E  G  T  V  N  N  H  H  F  K  C  T  S  E

                          SpAcc
                              |
               AGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGCGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTT
          701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 800
               TCCCGCTTCCGTTCGGGATGCTCCCGTGGGTCTGGTACTCTTAGTTCCGCCAGCTCCCGCCGGGAGAGGGGAAGCGGAAGCTGTAGGACCGATGGTCGAA
mKate2       >   G  E  G  K  P  Y  E  G  T  Q  T  M  R  I  K  A  V  E  G  G  P  L  P  F  A  F  D  I  L  A  T  S  F

                                                                               Bsu36       SpDon
                                                                                   |           |
               CATGTACGGCAGCAAAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACA
          801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 900
               GTACATGCCGTCGTTTTGGAAGTAGTTGGTGTGGGTCCCGTAGGGGCTGAAGAAATTCGTCAGGAAGGGACTCCCGAAGTGTACCCTCTCTCAGTGGTGT
mKate2       >  M  Y  G  S  K  T  F  I  N  H  T  Q  G  I  P  D  F  F  K  Q  S  F  P  E  G  F  T  W  E  R  V  T  T
```

```
                      Bbs1             Bpm1
                         |                |
               TACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCA
          901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1000
               ATGCTTCTGCCCCCGCACGACTGGCGATGGGTCCTGTGGTCGGAGGTCCTGCCGACGGAGTAGATGTTGCAGTTCTAGTCTCCCCACTTGAAGGGTAGGT
mKate2       > Y  E  D  G  G  V  L  T  A  T  Q  D  T  S  L  Q  D  G  C  L  I  Y  N  V  K  I  R  G  V  N  F  P  S  N

                                                     Bsa1
                                         BseY1    Stu1  |                                 Bgl1          Bgl1
                                             |       |  |                                    |             |
               ACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAGACCCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAGCCGACATGGCCCT
         1001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1100
               TGCCGGGACACTACGTCTTCTTTTGTGAGCCGACCCTCCGGAGGTGGCTCTGGGACATGGGGCGACTGCCGCCGGACCTTCCGTCTCGGCTGTACCGGGA
mKate2       >   G  P  V  M  Q  K  K  T  L  G  W  E  A  S  T  E  T  L  Y  P  A  D  G  G  L  E  G  R  A  D  M  A  L

               BssS1          Eco57                    Bbs1          BpuE1
                   |              |                       |              |
               GAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTTGAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTG
         1101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1200
               CTTCGAGCACCCGCCCCCGGTGGACTAGACGTTGAACTTCTGGTGTATGTCTAGGTTCTTTGGGCGATTCTTGGAGTTCTACGGGCCGCAGATGATACAC
mKate2       >  K  L  V  G  G  G  H  L  I  C  N  L  K  T  T  Y  R  S  K  K  P  A  K  N  L  K  M  P  G  V  Y  Y  V

                       Bbs1
                   SpAcc  |               Bsa1       PshA1       BssS1 Dra3      Msc1  AlwN1          SpAcc
                       |  |                  |           |           |    |         |      |              |
               GACAGAAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGG
         1201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1300
               CTGTCTTCTGACCTTTCTTAGTTCCTCCGGCTGTTTCTCTGGATGCAGCTCGTCGTGCTCCACCGACACCGGTCTATGACGCTGGAGGGATCGTTTGACC
mKate2       > D  R  R  L  E  R  I  K  E  A  D  K  E  T  Y  V  E  Q  H  E  V  A  V  A  R  Y  C  D  L  P  S  K  L  G

                                              Hind3
                            BpuE1     Xho1   Sac1 |       EcoR1
                 Bmr1    BspE1  | Bgl2   | Ecl2 | |   EcoRV   |   Nco1        AlwN1                    BseR1
                    |        |  |    |   |    | | |       |   |      |            |                        |
               GGCACAAACTTAATTCCGGATCAGATCTCGAGCTCAAGCTTGATATCGAATTCCCCATGGAGCAGAAGCTGATCTCCGAGGAGGACCTGAACCGGAAGAA
         1301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1400
               CCGTGTTTGAATTAAGGCCTAGTCTAGAGCTCGAGTTCGAACTATAGCTTAAGGGGTACCTCGTCTTCGACTAGAGGCTCCTCCTGGACTTGGCCTTCTT
mKate2/c-myc >   H  K  L  N  S  G  S  D  L  E  L  K  L  D  I  E  F  P  M  E  Q  K  L  I  S  E  E  D  L  N  R  K  K
```

```
                                  SpAcc        Eco57  Bbs1
                                      |            |     |
               ACTGGTGGTTGTTGGTGATGGAGCCTGTGGAAAGACATGCTTGCTCATAGTCTTCAGCAAGGACGAGTTCCCAGAGGTGTATGTGCCCACAGTGTTTGAG
         1401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1500
               TGACCACCAACAACCACTACCTCGGACACCTTTCTGTACGAACGAGTATCAGAAGTCGTTCCTGCTCAAGGGTCTCCACATACACGGGTGTCACAAACTC
GTFase       >  L  V  V  V  G  D  G  A  C  G  K  T  C  L  L  I  V  F  S  K  D  E  F  P  E  V  Y  V  P  T  V  F  E

                                  BspM1                                     Msc1                   Not1
                         EcoRV    BfuA1                                 Pvu2   |                   Eag1BsrB1
                             |        |                                    |   |                      |    |
               AACTATGTGGCAGATATCGAGGTGGATGGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGCCAGGAGGACTACGACCGCCTGCGGCCGCTCTCCT
         1501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1600
               TTGATACACCGTCTATAGCTCCACCTACCTTTCGTCCATCTCAACCGAAACACCCTGTGTCGACCGGTCCTCCTGATGCTGGCGGACGCCGGCGAGAGGA
GTFase       > N  Y  V  A  D  I  E  V  D  G  K  Q  V  E  L  A  L  W  D  T  A  G  Q  E  D  Y  D  R  L  R  P  L  S  Y

                           Aat2                                                        SanD1
                       Drd1   |                                                       Bpm1 |    SpDon   Xmn1
                          |   |                                                          | |        |      |
               ACCCGGACACCGACGTCATTCTCATGTGCTTCTCGGTGGACAGCCCGGACTCGCTGGAGAACATCCCCGAGAAGTGGGTCCCCGAGGTGAAGCACTTCTG
         1601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1700
               TGGGCCTGTGGCTGCAGTAAGAGTACACGAAGAGCCACCTGTCGGGCCTGAGCGACCTCTTGTAGGGGCTCTTCACCCAGGGGCTCCACTTCGTGAAGAC
GTFase       >   P  D  T  D  V  I  L  M  C  F  S  V  D  S  P  D  S  L  E  N  I  P  E  K  W  V  P  E  V  K  H  F  C

                                                          BspM1
                              Xcm1                        BfuA1
                             BstX1   Msc1              Fsp1   |    Drd1                                 Xmn1
                                 |      |                 |   |       |                                    |
               TCCCAATGTGCCCATCATCCTGGTGGCCAACAAAAAAGACCTGCGCAGCGACGAGCATGTCCGCACAGAGCTGGCCCGCATGAAGCAGGAACCCGTTCGC
         1701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1800
               AGGGTTACACGGGTAGTAGGACCACCGGTTGTTTTTTCTGGACGCGTCGCTGCTCGTACAGGCGTGTCTCGACCGGGCGTACTTCGTCCTTGGGCAAGCG
GTFase       >  P  N  V  P  I  I  L  V  A  N  K  K  D  L  R  S  D  E  H  V  R  T  E  L  A  R  M  K  Q  E  P  V  R

                                       Fsp1
                    Eag1    Nco1      FspA1       Bcg1a     Xho1                    Bcg1b   Bbs1  BsmB1
                       |       |          |           |        |                        |      |      |
               ACGGATGACGGCCGCGCCATGGCCGTGCGCATCCAAGCCTACGACTACCTCGAGTGCTCTGCCAAGACCAAGGAAGGCGTGCGCGAGGTCTTCGAGACGG
         1801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1900
               TGCCTACTGCCGGCGCGGTACCGGCACGCGTAGGTTCGGATGCTGATGGAGCTCACGAGACGGTTCTGGTTCCTTCCGCACGCGCTCCAGAAGCTCTGCC
GTFase       > T  D  D  G  R  A  M  A  V  R  I  Q  A  Y  D  Y  L  E  C  S  A  K  T  K  E  G  V  R  E  V  F  E  T  A
```

```
               BssH2    SpAcc Pst1 Afe1                       BstAP        SpAcc
                   |        |    |    |                           |            |
               CCACGCGCGCCGCGCTGCAGAAGCGCTACGGCTCCCAGAACGGCTGCATCAACTGCTGCAAGGTGCTATGAGGGCCGCGCCCGTCGCGCCTGCCCCTGCC
         1901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2000
               GGTGCGCGCGGCGCGACGTCTTCGCGATGCCGAGGGTCTTGCCGACGTAGTTGACGACGTTCCACGATACTCCCGGCGCGGGCAGCGCGGACGGGGACGG
GTFase       >   T  R  A  A  L  Q  K  R  Y  G  S  Q  N  G  C  I  N  C  C  K  V  L  *

             BamH1        Xba1      Bcl1BsaB1                            SpDon   Dra1
                 |           |         |    |                                |      |
               GGGGATCCACCGGATCTAGATAACTGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCT
         2001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2100
               CCCCTAGGTGGCCTAGATCTATTGACTAGTATTAGTCGGTATGGTGTAAACATCTCCAAAATGAACGAAATTTTTTGGAGGGTGTGGAGGGGGACTTGGA

                              Bsm1
                             Mfe1|        Hpa1       polyA    Psi1      polyA                        polyA
                                ||           |           |       |          |                            |
               GAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCA
         2101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2200
               CTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAATAACGTCGAATATTACCAATGTTTATTTCGTTATCGTAGTGTTTAAAGTGTTTATTTCGT

                  Bts1 Bsm1                                      Mlu1     SpDon        Ssp1
                     |    |                                         |         |           |
               TTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAAT
         2201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2300
               AAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGTTACATAGAATTGCGCATTTAACATTCGCAATTATAAAACAATTTTAAGCGCAATTTA

                                                                   Psi1
                                                                      |
               TTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAG
         2301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2400
               AAAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTAGCCGTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAACTCACAACAAGGTC

                                                                                               BtgZ1
                            BsaXb                 Drd1    BsaXa                                Dra3|
                                |                    |        |                                   ||
               TTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTA
         2401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2500
               AAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTGCAGTTTCCCGCTTTTTGGCAGATAGTCCCGCTACCGGGTGATGCACTTGGTAGTGGGAT
```

```
                                                                                         Nae1
                                                SpAcc                                 NgoM4 |
                                                    |                                     | |
      ATCAAGTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCG
2501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2600
      TAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGGGGGCTAAATCTCGAACTGCCCCTTTCGGCCGCTTGCACCGC


                             BsrB1
                                 |
      AGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGC
2601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2700
      TCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGACCGTTCACATCGCCAGTGCGACGCGCATTGGTGGTGTGGGCGGCGCGAATTACGCG


                                                                                                  BciV1
                                                                                               BspH1  |
                                                                 polyA                       BsrB1 |  |
                                                                     |                           | |  |
      CGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAG
2701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2800
      GCGATGTCCCGCGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTC


                                                                      Eci1
                               Ssp1  Ear1        Bsu36              Pvu2 |
                                  |     |            |                 | |
      ACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTCCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGT
2801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2900
      TGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCAGGACTCCGCCTTTCTTGGTCGACACCTTACACACAGTCAATCCCACACCTTTCA


                                          Nsi1
                                        Sph1 |
                                       BfrB1 |
            BseY1                   BstAP  | |            SexA1                     BseY1
                |                       |  | |                |                         |
      CCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTAT
2901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3000
      GGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATA
```

```
              Nsi1
            Sph1 |
           BfrB1 |                                                      Eci1
        BstAP  | |                       Eci1                 Eci1     Bmr1|       Eci1            Nco1
            |  | |                          |                    |        ||          |               |
      GCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCAT
3001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3100
      CGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGCGGGGTA

                                                                                            Avr2
                                             Sfi1                                          Stu1|
                      polyA                  Bgl1                    SpDon             BseR1  ||
                          |                     |                        |                 |  ||
      GGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTT
3101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3200
      CCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAGCCGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATCCGAAAA

                                                        BspM1
          Cla1              BsaB1                       BfuA1               Eag1
             |                  |                           |                  |
      GCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCG
3201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3300
      CGTTTCTAGCTAGTTCTCTGTCCTACTCCTAGCAAAGCGTACTAACTTGTTCTACCTAACGTGCGTCCAAGAGGCCGGCGAACCCACCTCTCCGATAAGC

                                                                      Nar1
                   Bmr1                                              Kas1|                       Drd1
                      |                                                 ||                          |
      GCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTC
3301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3400
      CGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGTCGCGTCCCCGCGGGCCAAGAAAAACAGTTCTGGCTGGACAG

                                                                            Pvu2
                                                    Msc1                Fsp1   |       PflF1
                                                       |                   |   |           |
      CGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCG
3401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3500
      GCCACGGGACTTACTTGACGTTCTGCTCCGTCGCGCCGATAGCACCGACCGGTGCTGCCCGCAAGGAACGCGTCGACACGAGCTGCAACAGTGACTTCGC
```

```
               Eco57                                      SpDon                         BciV1
                   |                                          |                             |
      GGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGC
3501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3600
      CCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAACGAGGACGGCTCTTTCATAGGTAGTACCGACTACGTTACG

                                        BtgZ1
                                     BspM1  |
  BsrD1                              BfuA1  |
      |                                  |  |
      GGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGA
3601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3700
      CCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCGTAGCTCGCTCGTGCATGAGCCTACCTTCGGCCAGAACAGCT

              Sap1
              Ear1                           BpuE1                         Sph1
                 |                               |                            |
      TCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTCGTGACC
3701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3800
      AGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCGAGTTCCGCTCGTACGGGCTGCCGCTCCTAGAGCAGCACTGG

                                                                         BseY1
                                                                        Nae1 |
  Nco1               BtgZ1                                           NgoM4 | |        Rsr2         Eci1
     |                   |                                               | | |           |            |
      CATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACA
3801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3900
      GTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTAGCTGACACCGGCCGACCCACACCGCCTGGCGATAGTCCTGT

                        Sap1                                                        BtgZ1
                        Ear1                   Eco57         BssS1                BsrB1 |
                           |                       |             |                    | |
      TAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCAT
3901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4000
      ATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCGCTTACCCGACTGGCGAAGGAGCACGAAATGCCATAGCGGCGAGGGCTAAGCGTCGCGTA
```

```
                                                                                       BssS1
                                                                                       BspM1
                                    BsrB1          BstB1                               BfuA1
                                        |              |                                   |
      CGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATT
4001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4100
      GCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCTTTACTGGCTGGTTCGCTGCGGGTTGGACGGTAGTGCTCTAAAGCTAA

                                                       Nae1
                                                    NgoM4 |
              SpAcc                                  Bpm1 |
                  |                                     | |
      CCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGC
4101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4200
      GGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAAGGCCCTGCGGCCGACCTACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAAGAAGCG

          Bpm1
      SpAcc  |
       Avr2  |                                                        polyA        polyA
          |  |                                                            |            |
      CCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGTGTT
4201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4300
      GGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTTATGGCCTTCCTTGGGCGCGATACTGCCGTTATTTTTCTGTCTTATTTTGCGTGCCACAA

                                                           Bsa1
                                                              |
      GGGTCGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTGGCACTCTGTCGATACCCCACCGAGACCCCATTGGGGCCAATACGCCCGCGTTTCTTCCT
4301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4400
      CCCAGCAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCGACCGTGAGACAGCTATGGGGTGGCTCTGGGGTAACCCCGGTTATGCGGGCGCAAAGAAGGA

                                                                      BstAP
                                                                      AlwN1      Bsu36
                                                                          |          |
      TTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGCCTCAGGTTACTCATATATACT
4401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4500
      AAAGGGGTGGGGTGGGGGGTTCAAGCCCACTTCCGGGTCCCGAGCGTCGGTTGCAGCCCCGCCGTCCGGGACGGTATCGGAGTCCAATGAGTATATATGA
```

```
              Dra1               Dra1                              BspH1
                 |                  |                                  |
      TTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTC
4501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4600
      AATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCCTAGATCCACTTCTAGGAAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAAGCAAG

                                                               BpuE1
                                                                   |
      CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTAC
4601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4700
      GTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAACTCTAGGAAAAAAAGACGCGCATTAGACGACGAACGTTTGTTTTTTTGGTGGCGATG

                                             Eco57                                               SpAcc
                                                 |                                                   |
      CAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTA
4701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4800
      GTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAGAAAAAGGCTTCCATTGACCGAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGATCACAT

                                                                              AlwN1
                                                                                  |
      GCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCG
4801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4900
      CGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGATGTATGGAGCGAGACGATTAGGACAATGGTCACCGACGACGGTCACCGCTATTCAGC

    BpuE1                                                            ApaL1     BseY1
        |                                                                |         |
      TGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGA
4901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5000
      ACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGTCGCCAGCCCGACTTGCCCCCCAAGCACGTGTGTCGGGTCGAACCTCGCTTGCT
```

```
                                                                                         BciV1
                                 SpAcc                                                Eci1   |
                                     |                                                   |   |
      CCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGG
5001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5100
      GGATGTGGCTTGACTCTATGGATGTCGCACTCGATACTCTTTCGCGGTGCGAAGGGCTTCCCTCTTTCCGCCTGTCCATAGGCCATTCGCCGTCCCAGCC

              BssS1                                    SpAcc                          Drd1
                  |                                        |                             |
      AACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA
5101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5200
      TTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCATAGAAATATCAGGACAGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAAAACACT

                                                                                        SpDon
  BpuE1               SpAcc     Eci1                                                   BspLU|
      |                   |        |                                                       ||
      TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTG
5201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5300
      ACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCAAGGACCGGAAAACGACCGGAAAACGAGTGTACAAGAAAGGAC

                                             Nsi1
                                          BfrB1 |
                                              | |
      CGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT
5301  |||||||||*|||||||||*|||||||||*|||||||||*|||| 5344
      GCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTACGTA
```

Found:

Aat2 Afe1 **Age1** **Ale1** AlwN1 ApaL1 **Ase1** Avr2 **BamH1** Bbs1 **Bcg1a** **Bcg1b** BciV1 **Bcl1**
BfrB1 BfuA1 Bgl1 **Bgl2** Bmr1 Bpm1 BpuE1 Bsa1 BsaB1 **BsaXa** **BsaXb** BseR1 BseY1 Bsm1
BsmB1 **BspE1** BspH1 **BspLU** BspM1 BsrB1 **BsrD1** **BsrG1** **BssH2** BssS1 BstAP **BstB1** **BstX1** Bsu36
BtgZ1 **Bts1** **Cla1** Dra1 Dra3 Drd1 Eag1 Ear1 Eci1 **Ecl2** Eco57 **EcoR1** EcoRV **FspA1**
Fsp1 **Hind3** **Hpa1** **Kas1** **Mfe1** **Mlu1** Msc1 Nae1 **Nar1** Nco1 **Nde1** NgoM4 **Nhe1** **Not1**
Nsi1 **PflF1** polyA **PshA1** Psi1 **Pst1** Pvu2 **Rsr2** **Sac1** **SanD1** Sap1 **SexA1** **Sfi1** **SnaB1**
SpAcc SpDon Sph1 Ssp1 Stu1 **Xba1** **Xcm1** Xho1 Xmn1

Unique:

Age1 **Ale1** **Ase1** **BamH1** **Bcg1a** **Bcg1b** **Bcl1** **Bgl2** **BsaXa** **BsaXb** **BsmB1** **BspE1** **BspLU** **BsrD1**
BsrG1 **BssH2** **BstB1** **BstX1** **Bts1** **Cla1** **Ecl2** **EcoR1** **FspA1** **Hind3** **Hpa1** **Kas1** **Mfe1** **Mlu1**
Nar1 **Nde1** **Nhe1** **Not1** **PflF1** **PshA1** **Pst1** **Rsr2** **Sac1** **SanD1** **SexA1** **Sfi1** **SnaB1** **Xba1**
Xcm1

Not found:

Aar1 Acc65 Acl1 Afl2 Ahd1 Apa1 Asc1 AsiS1 Bae1a Bae1b BbvC1 Blp1 BmgB1 Bpu10
Bsg1 BsiW1 BstE2 BstZ1 BxatB BxatL BxatR BxatP _Chi EcoK EcoN1 FCatB FCatL FCatR
FCatP ScFRT Fse1 I_Ceu Kpn1 loxP Nru1 Pac1 PflM1 Pme1 Pml1 PspOM Pvu1 R4atB
R4atL R4atP R4atR Sac2 Sal1 Sbf1 Sca1 Sgf1 SgrA1 Sma1 Spe1 Srf1 Swa1 T3RNA
T7RNA T7Ter PISce

Excluded by site complexity:

Acc1 Aci1 Afl3 Alu1 Alw1 Apo1 Ava1 Ava2 Ban1 Ban2 Bbv1 BceA1 Bfa1 Bme15
BsaA1 BsaH1 BsaJ1 BsaW1 BseM2 BsiE1 BsiH1 Bsl1 BsmA1 BsmF1 Bsp12 BspCa BspCb Bsr1
BsrF1 BssK1 BstF5 BstN1 BstU1 BstY1 Btg1 Cac8 CviJ1 Dde1 Eae1 EcoO1 Fau1 Fnu4H
Fok1 Hae2 Hae3 Hga1 Hha1 Hinc2 Hinf1 HinP1 Hpa2 Hph1 Hpy99 Hpy1 Hpy3 HpyC3
HpyC4 HpyC5 Mae3 Mbo2 Mnl1 Mse1 Msl1 MspA1 Mwo1 Nci1 Nla3 Nla4 Nsp1 Ple1
PpuM1 Rsa1 Sau3A Sau96 SfaN1 Sfc1 Sml1 Sty1 Taq1 Tat1 Tfi1 Tse1 Tsp45 Tsp50
TspR1