pmKate2-ER 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, endoplasmic reticulum (ER) targeting signal (calreticulin signal sequence) and ER retention signal (KDEL sequence) amino acids are shown in green, linker amino acids are shown in black.

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

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

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

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

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

                                                                                                      Nhe1    Sph1
                                                                                                         |       |
               ACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCATGC
          501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 600
               TGTTGAGGCGGGGTAACTGCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGACCAAATCACTTGGCAGTCTAGGCGATCGTACG
ER           >                                                                                                 M  L

                                                                           SpDon
                                          Sfi1               Age1         Ale1 |
                            BseR1         Bgl1             Rsr2 |       Nco1 | |
                                |            |                |  |          |  | |
               TGCTATCCGTGCCGTTGCTGCTCGGCCTCCTCGGCCTGGCCGTCGCCGGACCGGTCGCCACCATGGTGAGCGAGCTGATTAAGGAGAACATGCACATGAA
          601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 700
               ACGATAGGCACGGCAACGACGAGCCGGAGGAGCCGGACCGGCAGCGGCCTGGCCAGCGGTGGTACCACTCGCTCGACTAATTCCTCTTGTACGTGTACTT
ER/mKate2    >   L  S  V  P  L  L  L  G  L  L  G  L  A  V  A  G  P  V  A  T  M  V  S  E  L  I  K  E  N  M  H  M  K

             BsrG1                            ApaL1                     SpAcc
                 |                                    |                         |
               GCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGCG
          701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 800
               CGACATGTACCTCCCGTGGCACTTGTTGGTGGTGAAGTTCACGTGTAGGCTCCCGCTTCCGTTCGGGATGCTCCCGTGGGTCTGGTACTCTTAGTTCCGC
mKate2       >  L  Y  M  E  G  T  V  N  N  H  H  F  K  C  T  S  E  G  E  G  K  P  Y  E  G  T  Q  T  M  R  I  K  A

               GTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAAAACCTTCATCAACCACACCCAGGGCATCCCCGACT
          801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 900
               CAGCTCCCGCCGGGAGAGGGGAAGCGGAAGCTGTAGGACCGATGGTCGAAGTACATGCCGTCGTTTTGGAAGTAGTTGGTGTGGGTCCCGTAGGGGCTGA
mKate2       > V  E  G  G  P  L  P  F  A  F  D  I  L  A  T  S  F  M  Y  G  S  K  T  F  I  N  H  T  Q  G  I  P  D  F

                                         SpDon                          Bbs1             Bpm1
                                             |                             |                |
               TCTTTAAGCAGTCCTTCCCCGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGA
          901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1000
               AGAAATTCGTCAGGAAGGGGCTCCCGAAGTGTACCCTCTCTCAGTGGTGTATGCTTCTGCCCCCGCACGACTGGCGATGGGTCCTGTGGTCGGAGGTCCT
mKate2       >   F  K  Q  S  F  P  E  G  F  T  W  E  R  V  T  T  Y  E  D  G  G  V  L  T  A  T  Q  D  T  S  L  Q  D
```

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                                                                                                       Bsa1
                                                                                           BseY1    Stu1  |
                                                                                               |       |  |
               CGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAG
         1001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1100
               GCCGACGGAGTAGATGTTGCAGTTCTAGTCTCCCCACTTGAAGGGTAGGTTGCCGGGACACTACGTCTTCTTTTGTGAGCCGACCCTCCGGAGGTGGCTC
mKate2       >  G  C  L  I  Y  N  V  K  I  R  G  V  N  F  P  S  N  G  P  V  M  Q  K  K  T  L  G  W  E  A  S  T  E

                                        Bgl1          Bgl1       BssS1          Eco57                    Bbs1
                                           |             |           |              |                       |
               ACCCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAGCCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTTGAAGACCACATACA
         1101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1200
               TGGGACATGGGGCGACTGCCGCCGGACCTTCCGTCTCGGCTGTACCGGGACTTCGAGCACCCGCCCCCGGTGGACTAGACGTTGAACTTCTGGTGTATGT
mKate2       > T  L  Y  P  A  D  G  G  L  E  G  R  A  D  M  A  L  K  L  V  G  G  G  H  L  I  C  N  L  K  T  T  Y  R

                                                                         Bbs1
                   BpuE1                                             SpAcc  |               Bsa1       PshA1
                       |                                                 |  |                  |           |
               GATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACAGAAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACCTACGTCGA
         1201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1300
               CTAGGTTCTTTGGGCGATTCTTGGAGTTCTACGGGCCGCAGATGATACACCTGTCTTCTGACCTTTCTTAGTTCCTCCGGCTGTTTCTCTGGATGCAGCT
mKate2       >   S  K  K  P  A  K  N  L  K  M  P  G  V  Y  Y  V  D  R  R  L  E  R  I  K  E  A  D  K  E  T  Y  V  E

                                                                                        BseR1
                                                                    Ale1            SpAcc   |
               BssS1 Dra3      Msc1  AlwN1          SpAcc          Bmr1|          Bgl2  |   |     SpAcc  Hind3
                   |    |         |      |              |             ||            |  |   |         |      |
               GCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGGCACAGAGGTGGAGGAGGTAGATCTGGAAAGGACGAGCTGTAAAAGCTT
         1301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1400
               CGTCGTGCTCCACCGACACCGGTCTATGACGCTGGAGGGATCGTTTGACCCCGTGTCTCCACCTCCTCCATCTAGACCTTTCCTGCTCGACATTTTCGAA
mKate2       >  Q  H  E  V  A  V  A  R  Y  C  D  L  P  S  K  L  G  H  R  G  G  G  G  R  S  G  K  D  E  L  *
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                                  BamH1
                                 Sma1 |
                                Apa1| |
                           PspOM   || |
                           Sac2|   || |
   EcoR1      Sal1      Kpn1  ||   || |
 BstB1 |     Pst1| Acc65   |  ||   || |        Xba1      Bcl1BsaB1                            SpDon
     | |        ||     |   |  ||   || |           |         |    |                                |
      CGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGATCTAGATAACTGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTT
1401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1500
      GCTTAAGACGTCAGCTGCCATGGCGCCCGGGCCCTAGGTGGCCTAGATCTATTGACTAGTATTAGTCGGTATGGTGTAAACATCTCCAAAATGAACGAAA


                                                   Bsm1
  Dra1                                            Mfe1|        Hpa1       polyA    Psi1      polyA
     |                                               ||           |           |       |          |
      AAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGC
1501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1600
      TTTTTTGGAGGGTGTGGAGGGGGACTTGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAATAACGTCGAATATTACCAATGTTTATTTCG


                      polyA            Bts1 Bsm1                                      Mlu1     SpDon
                          |               |    |                                         |         |
      AATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGTAAATTGTAAGC
1601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1700
      TTATCGTAGTGTTTAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGTTACATAGAATTGCGCATTTAACATTCG


        Ssp1                                                                            Psi1
           |                                                                               |
      GTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAAT
1701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1800
      CAATTATAAAACAATTTTAAGCGCAATTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTAGCCGTTTTAGGGAATATTTAGTTTTCTTA


                                                 BsaXb                Drd1    BsaXa
                                                     |                    |        |
      AGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGG
1801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1900
      TCTGGCTCTATCCCAACTCACAACAAGGTCAAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTGCAGTTTCCCGCTTTTTGGCAGATAGTCCC
```

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                BtgZ1
                Dra3|                                                         SpAcc
                   ||                                                             |
      CGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGA
1901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2000
      GCTACCGGGTGATGCACTTGGTAGTGGGATTAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGGGGGCTAAATCT


                   Nae1
                NgoM4 |                                    BsrB1
                    | |                                        |
      GCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCG
2001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2100
      CGAACTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGACCGTTCACATCGCCAGTGCGACGCGC


                                                                                               polyA
                                                                                                   |
      TAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTA
2101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2200
      ATTGGTGGTGTGGGCGGCGCGAATTACGCGGCGATGTCCCGCGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAAGAT


                            BciV1
                         BspH1  |                                                                   Eci1
                       BsrB1 |  |                            Ssp1  Ear1        Bsu36              Pvu2 |
                           | |  |                               |     |            |                 | |
      AATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTCCTGAGGCGGAAAGAACCAGCTGT
2201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2300
      TTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCAGGACTCCGCCTTTCTTGGTCGACA


                                                                        Nsi1
                                                                      Sph1 |
                                                                     BfrB1 |
                                          BseY1                   BstAP  | |            SexA1
                                              |                       |  | |                |
      GGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAA
2301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2400
      CCTTACACACAGTCAATCCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTT
```

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                                            Nsi1
                                          Sph1 |
                                         BfrB1 |
              BseY1                   BstAP  | |                       Eci1                 Eci1     Bmr1
                  |                       |  | |                          |                    |        |
      GTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACT
2401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2500
      CAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGA

                                                                           Sfi1
  Eci1       Eci1           Nco1                   polyA                  Bgl1                    SpDon
     |          |               |                       |                     |                        |
      CCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGT
2501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2600
      GGCGGGTCAAGGCGGGTAAGAGGCGGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAGCCGGAGACTCGATAAGGTCTTCATCA

                      Avr2
                     Stu1|                                                            BspM1
                 BseR1  ||              Cla1              BsaB1                       BfuA1
                     |  ||                 |                  |                           |
      GAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTT
2601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2700
      CTCCTCCGAAAAAACCTCCGGATCCGAAAACGTTTCTAGCTAGTTCTCTGTCCTACTCCTAGCAAAGCGTACTAACTTGTTCTACCTAACGTGCGTCCAA

                                                                                                    Nar1
      Eag1                                       Bmr1                                              Kas1|
         |                                          |                                                 ||
      CTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCG
2701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2800
      GAGGCCGGCGAACCCACCTCTCCGATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGTCGCGTCCCCGC

                           Drd1                                                   Msc1
                              |                                                      |
      CCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGC
2801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2900
      GGGCCAAGAAAAACAGTTCTGGCTGGACAGGCCACGGGACTTACTTGACGTTCTGCTCCGTCGCGCCGATAGCACCGACCGGTGCTGCCCGCAAGGAACG
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      Pvu2
  Fsp1   |       PflF1                       Eco57                                      SpDon
     |   |           |                           |                                          |
      GCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCG
2901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3000
      CGTCGACACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAACGAGGACGGC


                                                                      BtgZ1
                                                                   BspM1  |
                  BciV1         BsrD1                              BfuA1  |
                      |             |                                  |  |
      AGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGC
3001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3100
      TCTTTCATAGGTAGTACCGACTACGTTACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCGTAGCTCGCTCG


                                            Sap1
                                            Ear1                           BpuE1
                                               |                               |
      ACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGC
3101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3200
      TGCATGAGCCTACCTTCGGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCGAGTTCCGCTCG


     Sph1                       Nco1               BtgZ1                                           NgoM4
        |                          |                   |                                               |
      ATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCC
3201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3300
      TACGGGCTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTAGCTGACACCGG


   BseY1                                              Sap1
  Nae1 |        Rsr2         Eci1                     Ear1                   Eco57         BssS1
     | |           |            |                        |                       |             |
      GGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTA
3301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3400
      CCGACCCACACCGCCTGGCGATAGTCCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCGCTTACCCGACTGGCGAAGGAGCACGAAAT
```

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              BtgZ1
            BsrB1 |                                               BsrB1          BstB1
                | |                                                   |              |
      CGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGA
3401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3500
      GCCATAGCGGCGAGGGCTAAGCGTCGCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCTTTACTGGCTGGTTCGCT

                 BssS1                                                               Nae1
                 BspM1                                                            NgoM4 |
                 BfuA1                      SpAcc                                  Bpm1 |
                     |                          |                                     | |
      CGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCA
3501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3600
      GCGGGTTGGACGGTAGTGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAAGGCCCTGCGGCCGACCTACTAGGAGGT

                                        Bpm1
                                    SpAcc  |
                                     Avr2  |                                                        polyA
                                        |  |                                                            |
      GCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCA
3601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3700
      CGCGCCCCTAGAGTACGACCTCAAGAAGCGGGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTTATGGCCTTCCTTGGGCGCGATACTGCCGT

             polyA                                                                   Bsa1
                 |                                                                      |
      ATAAAAAGACAGAATAAAACGCACGGTGTTGGGTCGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTGGCACTCTGTCGATACCCCACCGAGACCCC
3701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3800
      TATTTTTCTGTCTTATTTTGCGTGCCACAACCCAGCAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCGACCGTGAGACAGCTATGGGGTGGCTCTGGGG

                                                                                                    BstAP
                                                                                                    AlwN1
                                                                                                        |
      ATTGGGGCCAATACGCCCGCGTTTCTTCCTTTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCC
3801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3900
      TAACCCCGGTTATGCGGGCGCAAAGAAGGAAAAGGGGTGGGGTGGGGGGTTCAAGCCCACTTCCGGGTCCCGAGCGTCGGTTGCAGCCCCGCCGTCCGGG
```

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           Bsu36                            Dra1               Dra1                              BspH1
               |                               |                  |                                  |
      TGCCATAGCCTCAGGTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATG
3901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4000
      ACGGTATCGGAGTCCAATGAGTATATATGAAATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCCTAGATCCACTTCTAGGAAAAACTATTAGAGTAC

                                                                                             BpuE1
                                                                                                 |
      ACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCT
4001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4100
      TGGTTTTAGGGAATTGCACTCAAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAACTCTAGGAAAAAAAGACGCGCATTAGA

                                                                           Eco57
                                                                               |
      GCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGC
4101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4200
      CGACGAACGTTTGTTTTTTTGGTGGCGATGGTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAGAAAAAGGCTTCCATTGACCGAAGTCGTCTCG

                           SpAcc
                               |
      GCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTA
4201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4300
      CGTCTATGGTTTATGACAGGAAGATCACATCGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGATGTATGGAGCGAGACGATTAGGACAAT

        AlwN1                     BpuE1
            |                         |
      CCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTT
4301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4400
      GGTCACCGACGACGGTCACCGCTATTCAGCACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGTCGCCAGCCCGACTTGCCCCCCAA

   ApaL1     BseY1                                             SpAcc
       |         |                                                 |
      CGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGC
4401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4500
      GCACGTGTGTCGGGTCGAACCTCGCTTGCTGGATGTGGCTTGACTCTATGGATGTCGCACTCGATACTCTTTCGCGGTGCGAAGGGCTTCCCTCTTTCCG
```

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                   BciV1
                Eci1   |                    BssS1                                    SpAcc
                   |   |                        |                                        |
      GGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGC
4501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4600
      CCTGTCCATAGGCCATTCGCCGTCCCAGCCTTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCATAGAAATATCAGGACAGCCCAAAGCG


                Drd1            BpuE1               SpAcc     Eci1
                   |                |                   |        |
      CACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTT
4601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4700
      GTGGAGACTGAACTCGCAGCTAAAAACACTACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCAAGGACCGGAAAA


                  SpDon                                                    Nsi1
                 BspLU|                                                 BfrB1 |
                     ||                                                     | |
      GCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT
4701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||| 4774
      CGACCGGAAAACGAGTGTACAAGAAAGGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTACGTA
```

```
Found:

    Aat2   Acc65    Age1    Ale1   AlwN1    Apa1   ApaL1    Ase1    Avr2   BamH1    Bbs1   BciV1    Bcl1   BfrB1
   BfuA1    Bgl1    Bgl2    Bmr1    Bpm1   BpuE1    Bsa1   BsaB1   BsaXa   BsaXb   BseR1   BseY1    Bsm1   BspH1
   BspLU   BspM1   BsrB1   BsrD1   BsrG1   BssS1   BstAP   BstB1   Bsu36   BtgZ1    Bts1    Cla1    Dra1    Dra3
    Drd1    Eag1    Ear1    Eci1   Eco57   EcoR1    Fsp1   Hind3    Hpa1    Kas1    Kpn1    Mfe1    Mlu1    Msc1
    Nae1    Nar1    Nco1    Nde1   NgoM4    Nhe1    Nsi1   PflF1   polyA   PshA1    Psi1   PspOM    Pst1    Pvu2
    Rsr2    Sac2    Sal1    Sap1   SexA1    Sfi1    Sma1   SnaB1   SpAcc   SpDon    Sph1    Ssp1    Stu1    Xba1

Unique:

   Acc65    Age1    Apa1    Ase1   BamH1    Bcl1    Bgl2   BsaXa   BsaXb   BspLU   BsrD1   BsrG1    Bts1    Cla1
    Eag1   EcoR1    Fsp1   Hind3    Hpa1    Kas1    Kpn1    Mfe1    Mlu1    Nar1    Nde1    Nhe1   PflF1   PshA1
   PspOM    Pst1    Sac2    Sal1   SexA1    Sma1   SnaB1    Xba1


Not found:

    Aar1    Acl1    Afe1    Afl2    Ahd1    Asc1   AsiS1   Bae1a   Bae1b   BbvC1   Bcg1a   Bcg1b    Blp1   BmgB1
   Bpu10    Bsg1   BsiW1   BsmB1   BspE1   BssH2   BstE2   BstX1   BstZ1   BxatB   BxatL   BxatR   BxatP    _Chi
    Ecl2    EcoK   EcoN1   EcoRV   FCatB   FCatL   FCatR   FCatP   ScFRT    Fse1   FspA1   I_Ceu    loxP    Not1
    Nru1    Pac1   PflM1    Pme1    Pml1    Pvu1   R4atB   R4atL   R4atP   R4atR    Sac1   SanD1    Sbf1    Sca1
    Sgf1   SgrA1    Spe1    Srf1    Swa1   T3RNA   T7RNA   T7Ter   PISce    Xcm1    Xho1    Xmn1

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
```