**pmKate2-EB3** 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 base pairs 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, EB3 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
```

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


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


                          Hind3
                  Xho1    Sac1 |  EcoR1      Sal1         Bgl1
    BpuE1 Bgl2    | Ecl2 | |BstB1 |      Pst1|       Nco1 |                    Ale1
        |    |    |    | | |    | |        ||          | |                      |
     CCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACTGCCACCATGGCCGTCAATGTGTACTCCACATCTGTGACCAGTGAAAATCTGAGTC
601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 700
     GGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCAGCTGACGGTGGTACCGGCAGTTACACATGAGGTGTAGACACTGGTCACTTTTAGACTCAG
EB3     >                                                M  A  V  N  V  Y  S  T  S  V  T  S  E  N  L  S  R

                       Bsg1                                                                      PflM1
                         |                                                                          |
     GCCATGATATGCTTGCATGGGTCAACGACTCCCTGCACCTCAACTATACCAAGATAGAACAGCTTTGTTCAGGGGCAGCCTACTGCCAGTTCATGGACAT
701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 800
     CGGTACTATACGAACGTACCCAGTTGCTGAGGGACGTGGAGTTGATATGGTTCTATCTTGTCGAAACAAGTCCCCGTCGGATGACGGTCAAGTACCTGTA
EB3     >  H  D  M  L  A  W  V  N  D  S  L  H  L  N  Y  T  K  I  E  Q  L  C  S  G  A  A  Y  C  Q  F  M  D  M

            Sap1
            Ear1    ApaL1         SpDon       BpuE1                                   EcoK
               |        |             |           |                                      |
     GCTCTTCCCCGGCTGTGTGCACTTGAGGAAAGTGAAGTTCCAGGCCAAACTAGAGCATGAATACATCCACAACTTCAAGGTGCTGCAAGCAGCTTTCAAG
801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 900
     CGAGAAGGGGCCGACACACGTGAACTCCTTTCACTTCAAGGTCCGGTTTGATCTCGTACTTATGTAGGTGTTGAAGTTCCACGACGTTCGTCGAAAGTTC
EB3     >  L  F  P  G  C  V  H  L  R  K  V  K  F  Q  A  K  L  E  H  E  Y  I  H  N  F  K  V  L  Q  A  A  F  K
```

```
                                SpAcc SpAcc                                        polyA
                                    |     |                                          |
            AAGATGGGTGTTGACAAAATCATTCCTGTAGAGAAATTAGTGAAAGGAAAATTCCAAGATAATTTTGAGTTTATTCAGTGGTTTAAGAAATTCTTTGACG
       901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1000
            TTCTACCCACAACTGTTTTAGTAAGGACATCTCTTTAATCACTTTCCTTTTAAGGTTCTATTAAAACTCAAATAAGTCACCAAATTCTTTAAGAAACTGC
EB3       > K  M  G  V  D  K  I  I  P  V  E  K  L  V  K  G  K  F  Q  D  N  F  E  F  I  Q  W  F  K  K  F  F  D  A

                                                                          EcoN1   Bcl1 Bgl2
                                                                              |      |    |
            CAAACTATGATGGAAAGGATTACAACCCTCTGCTGGCGCGGCAGGGCCAGGACGTAGCGCCACCTCCTAACCCAGGTGATCAGATCTTCAACAAATCCAA
      1001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1100
            GTTTGATACTACCTTTCCTAATGTTGGGAGACGACCGCGCCGTCCCGGTCCTGCATCGCGGTGGAGGATTGGGTCCACTAGTCTAGAAGTTGTTTAGGTT
EB3       >   N  Y  D  G  K  D  Y  N  P  L  L  A  R  Q  G  Q  D  V  A  P  P  P  N  P  G  D  Q  I  F  N  K  S  K

                                                                                 Blp1
                                                                             Nae1  |
                                      Aat2    SpAcc                     NgoM4 |  |      BsrD1       Bsm1
                                         |       |                        | |  |          |          |
            GAAACTCATTGGCACAGCAGTTCCACAGAGGACGTCCCCCACAGGCCCAAAAAACATGCAGACCTCTGGCCGGCTGAGCAATGTGGCCCCCCCCTGCATT
      1101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1200
            CTTTGAGTAACCGTGTCGTCAAGGTGTCTCCTGCAGGGGGTGTCCGGGTTTTTTGTACGTCTGGAGACCGGCCGACTCGTTACACCGGGGGGGGACGTAA
EB3       >  K  L  I  G  T  A  V  P  Q  R  T  S  P  T  G  P  K  N  M  Q  T  S  G  R  L  S  N  V  A  P  P  C  I

                                                                                  Pvu2
          BspE1                                                                   PflM1   _Chi
             |                                                                       |      |
            CTCCGGAAGAATCCTCCATCAGCCCGAAATGGCGGCCATGAGACTGATGCCCAAATTCTTGAACTCAACCAACAGCTGGTGGACTTGAAGCTGACAGTGG
      1201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1300
            GAGGCCTTCTTAGGAGGTAGTCGGGCTTTACCGCCGGTACTCTGACTACGGGTTTAAGAACTTGAGTTGGTTGTCGACCACCTGAACTTCGACTGTCACC
EB3       > L  R  K  N  P  P  S  A  R  N  G  G  H  E  T  D  A  Q  I  L  E  L  N  Q  Q  L  V  D  L  K  L  T  V  D

                                                                  Sac1
            SpAcc    Eco57         Bpm1                       Ecl2 |                SpDon
                |        |            |                          | |                   |
            ATGGGCTGGAGAAGGAACGTGACTTCTACTTCAGCAAACTTCGTGACATCGAGCTCATCTGCCAGGAGCATGAAAGTGAAAACAGCCCTGTTATCTCAGG
      1301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1400
            TACCCGACCTCTTCCTTGCACTGAAGATGAAGTCGTTTGAAGCACTGTAGCTCGAGTAGACGGTCCTCGTACTTTCACTTTTGTCGGGACAATAGAGTCC
EB3       >   G  L  E  K  E  R  D  F  Y  F  S  K  L  R  D  I  E  L  I  C  Q  E  H  E  S  E  N  S  P  V  I  S  G
```

```
                                                   Sap1
                                         Bsu36     Ear1                    Bbs1      BamH1
                                           |         |                       |         |
          CATCATTGGCATCCTCTATGCCACAGAGGAAGGATTCGCACCCCCTGAGGACGATGAGATTGAAGAGCATCAACAAGAAGACCAGGACGAGTACCTGGAT
     1401 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1500
          GTAGTAACCGTAGGAGATACGGTGTCTCCTTCCTAAGCGTGGGGGACTCCTGCTACTCTAACTTCTCGTAGTTGTTCTTCTGGTCCTGCTCATGGACCTA
EB3          >  I  I  G  I  L  Y  A  T  E  E  G  F  A  P  P  E  D  D  E  I  E  E  H  Q  Q  E  D  Q  D  E  Y  L  D

                        SpDon
                       Ale1 |
          Age1       Nco1 | |                          BsrG1                          ApaL1
            |          | | |                             |                              |
          CCACCGGTCGCCACCATGGTGAGCGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACAT
     1501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1600
          GGTGGCCAGCGGTGGTACCACTCGCTCGACTAATTCCTCTTGTACGTGTACTTCGACATGTACCTCCCGTGGCACTTGTTGGTGGTGAAGTTCACGTGTA
mKate2       > P  P  V  A  T  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

                        SpAcc
                           |
          CCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGCGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAG
     1601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1700
          GGCTCCCGCTTCCGTTCGGGATGCTCCCGTGGGTCTGGTACTCTTAGTTCCGCCAGCTCCCGCCGGGAGAGGGGAAGCGGAAGCTGTAGGACCGATGGTC
mKate2       >   E  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

                                                                                   SpDon
                                                                                      |
          CTTCATGTACGGCAGCAAAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCCGAGGGCTTCACATGGGAGAGAGTCACC
     1701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1800
          GAAGTACATGCCGTCGTTTTGGAAGTAGTTGGTGTGGGTCCCGTAGGGGCTGAAGAAATTCGTCAGGAAGGGGCTCCCGAAGTGTACCCTCTCTCAGTGG
mKate2       >  F  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

                     Bbs1              Bpm1
                       |                 |
          ACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCAT
     1801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1900
          TGTATGCTTCTGCCCCCGCACGACTGGCGATGGGTCCTGTGGTCGGAGGTCCTGCCGACGGAGTAGATGTTGCAGTTCTAGTCTCCCCACTTGAAGGGTA
mKate2       > T  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
```

```
                                                Bsa1
                                     BseY1    Stu1  |                                  Bgl1          Bgl1
                                         |        |  |                                     |             |
            CCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAGACCCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAGCCGACATGGC
      1901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2000
            GGTTGCCGGGACACTACGTCTTCTTTTGTGAGCCGACCCTCCGGAGGTGGCTCTGGGACATGGGGCGACTGCCGCCGGACCTTCCGTCTCGGCTGTACCG
mKate2        >  N  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

                 BssS1          Eco57                    Bbs1          BpuE1
                     |              |                       |             |
            CCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTTGAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTAT
      2001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2100
            GGACTTCGAGCACCCGCCCCCGGTGGACTAGACGTTGAACTTCTGGTGTATGTCTAGGTTCTTTGGGCGATTCTTGGAGTTCTACGGGCCGCAGATGATA
mKate2        >  L  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

                       Bbs1
                 SpAcc  |                Bsa1       PshA1       BssS1 Dra3      Msc1  AlwN1          SpAcc
                     |  |                  |           |           |    |         |      |             |
            GTGGACAGAAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAAC
      2101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2200
            CACCTGTCTTCTGACCTTTCTTAGTTCCTCCGGCTGTTTCTCTGGATGCAGCTCGTCGTGCTCCACCGACACCGGTCTATGACGCTGGAGGGATCGTTTG
mKate2        > V  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

                       Not1
                       Eag1
                 Bmr1 BsrB1|      Xba1   BsaB1                            SpDon   Dra1
                     |     ||         |       |                                |      |
            TGGGGCACAGATGAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGA
      2201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2300
            ACCCCGTGTCTACTCGCCGGCGCTGAGATCTAGTATTAGTCGGTATGGTGTAAACATCTCCAAAATGAACGAAATTTTTTGGAGGGTGTGGAGGGGGACT
mKate2        >  G  H  R  *

                          Bsm1
                         Mfe1|        Hpa1       polyA    Psi1      polyA                        polyA
                            ||           |           |       |          |                            |
            ACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAA
      2301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2400
            TGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAATAACGTCGAATATTACCAATGTTTATTTCGTTATCGTAGTGTTTAAAGTGTTTATT
```

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            Bts1 Bsm1                                   Afl2         SpDon        Ssp1
                |    |                                     |             |           |
     AGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAAGGCGTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTT
2401 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2500
     TCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGTTACATAGAATTCCGCATTTAACATTCGCAATTATAAAACAATTTTAAGCGCAA

                                                                  Psi1
                                                                    |
     AAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTT
2501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2600
     TTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTAGCCGTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAACTCACAACAA

                                                                                          BtgZ1
                      BsaXb                    Drd1    BsaXa                              Dra3|
                          |                       |        |                                  ||
     CCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCAC
2601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2700
     GGTCAAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTGCAGTTTCCCGCTTTTTGGCAGATAGTCCCGCTACCGGGTGATGCACTTGGTAGTG

                                                                                             Nae1
                                                  SpAcc                                   NgoM4 |
                                                     |                                        | |
     CCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGT
2701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2800
     GGATTAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGGGGGCTAAATCTCGAACTGCCCCTTTCGGCCGCTTGCA

                                BsrB1
                                   |
     GGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAAT
2801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2900
     CCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGACCGTTCACATCGCCAGTGCGACGCGCATTGGTGGTGTGGGCGGCGCGAATTA

                                                                                                  BspH1
                                                                          polyA                 BsrB1 |
                                                                            |                       | |
     GCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCA
2901 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3000
     CGCGGCGATGTCCCGCGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGT
```

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                                                                  Eci1
  BciV1                           Ssp1  Ear1        Bsu36        Pvu2 |
      |                              |     |            |            | |
      TGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTCCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGA
3001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3100
      ACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCAGGACTCCGCCTTTCTTGGTCGACACCTTACACACAGTCAATCCCACACCT

                                          Nsi1
                                        Sph1 |
                                       BfrB1 |
                BseY1                BstAP  | |          SexA1                    BseY1
                    |                   |  | |             |                        |
      AAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAA
3101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3200
      TTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTT

                  Nsi1
                Sph1 |
               BfrB1 |                                                      Eci1
           BstAP  | |                      Eci1                 Eci1     Bmr1|       Eci1
                |  | |                         |                    |        ||          |
      GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCC
3201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3300
      CATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGCGG

                                                                                                  Avr2
                                                 Sfi1                                            Stu1|
   Nco1                   polyA                  Bgl1                      SpDon             BseR1  ||
      |                      |                     |                          |                 |  ||
      CCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGC
3301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3400
      GGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAGCCGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATCCG

                                                        BspM1
              Cla1                BsaB1                 BfuA1                Eag1
                |                    |                     |                    |
      TTTTGCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTA
3401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3500
      AAAACGTTTCTAGCTAGTTCTCTGTCCTACTCCTAGCAAAGCGTACTAACTTGTTCTACCTAACGTGCGTCCAAGAGGCCGGCGAACCCACCTCTCCGAT
```

```
                                                                  Nar1
                   Bmr1                                          Kas1|                        Drd1
                      |                                             ||                          |
     TTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACC
3501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3600
     AAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGTCGCGTCCCCGCGGGCCAAGAAAAACAGTTCTGGCTGG

                                                                        Pvu2
                                                   Msc1                Fsp1   |       PflF1
                                                      |                   |   |           |
     TGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGA
3601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3700
     ACAGGCCACGGGACTTACTTGACGTTCTGCTCCGTCGCGCCGATAGCACCGACCGGTGCTGCCCGCAAGGAACGCGTCGACACGAGCTGCAACAGTGACT

                 Eco57                                      SpDon                        BciV1
                    |                                          |                            |
     AGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCA
3701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3800
     TCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAACGAGGACGGCTCTTTCATAGGTAGTACCGACTACGT

                                       BtgZ1
                                    BspM1  |
     BsrD1                          BfuA1  |
        |                              |   |
     ATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTG
3801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3900
     TACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCGTAGCTCGCTCGTGCATGAGCCTACCTTCGGCCAGAAC

                Sap1
                Ear1                          BpuE1                        Sph1
                   |                             |                            |
     TCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTCGT
3901 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4000
     AGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCGAGTTCCGCTCGTACGGGCTGCCGCTCCTAGAGCAGCA
```

```
                                                                  BseY1
                                                                 Nae1 |
      Nco1               BtgZ1                                NgoM4 | |        Rsr2
         |                  |                                     | | |           |
      GACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAG
4001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4100
      CTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTAGCTGACACCGGCCGACCCACACCGCCTGGCGATAGTC

                          Sap1                                                          BtgZ1
   Eci1                   Ear1                   Eco57         BssS1                 BsrB1 |
      |                      |                      |             |                     | |
      GACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGC
4101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4200
      CTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCGCTTACCCGACTGGCGAAGGAGCACGAAATGCCATAGCGGCGAGGGCTAAGCGTCG

                                                                                          BssS1
                                                                                          BspM1
                                   BsrB1          BstB1                                  BfuA1
                                      |              |                                       |
      GCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTC
4201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4300
      CGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCTTTACTGGCTGGTTCGCTGCGGGTTGGACGGTAGTGCTCTAAAG

                                                   Nae1
                                                 NgoM4 |
                SpAcc                             Bpm1 |
                   |                                 | |
      GATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCT
4301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4400
      CTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAAGGCCCTGCGGCCGACCTACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAAGA

            Bpm1
         SpAcc  |
          Avr2  |                                                        polyA        polyA
            |   |                                                          |            |
      TCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGG
4401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4500
      AGCGGGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTTATGGCCTTCCTTGGGCGCGATACTGCCGTTATTTTTCTGTCTTATTTTGCGTGCC
```

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                                                          Bsa1
                                                            |
     TGTTGGGTCGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTGGCACTCTGTCGATACCCCACCGAGACCCCATTGGGGCCAATACGCCCGCGTTTCT
4501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4600
     ACAACCCAGCAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCGACCGTGAGACAGCTATGGGGTGGCTCTGGGGTAACCCCGGTTATGCGGGCGCAAAGA

                                                                  BstAP
                                                                  AlwN1      Bsu36
                                                                     |          |
     TCCTTTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGCCTCAGGTTACTCATATA
4601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4700
     AGGAAAAGGGGTGGGGTGGGGGGTTCAAGCCCACTTCCGGGTCCCGAGCGTCGGTTGCAGCCCCGCCGTCCGGGACGGTATCGGAGTCCAATGAGTATAT

                Dra1                Dra1                                BspH1
                  |                   |                                   |
     TACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTC
4701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4800
     ATGAAATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCCTAGATCCACTTCTAGGAAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAAG

                                                                  BpuE1
                                                                    |
     GTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCG
4801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4900
     CAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAACTCTAGGAAAAAAAGACGCGCATTAGACGACGAACGTTTGTTTTTTTGGTGGC

                                                Eco57
                                                  |
     CTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAG
4901 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5000
     GATGGTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAGAAAAAGGCTTCCATTGACCGAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGATC

 SpAcc                                                                          AlwN1
    |                                                                              |
     TGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAA
5001 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5100
     ACATCGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGATGTATGGAGCGAGACGATTAGGACAATGGTCACCGACGACGGTCACCGCTATT
```

```
        BpuE1                                                          ApaL1     BseY1
            |                                                              |         |
     GTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGA
5101 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5200
     CAGCACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGTCGCCAGCCCGACTTGCCCCCCAAGCACGTGTGTCGGGTCGAACCTCGCT

                                                                                                  BciV1
                                 SpAcc                                                         Eci1   |
                                     |                                                            |   |
     ACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGG
5201 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5300
     TGCTGGATGTGGCTTGACTCTATGGATGTCGCACTCGATACTCTTTCGCGGTGCGAAGGGCTTCCCTCTTTCCGCCTGTCCATAGGCCATTCGCCGTCCC

                BssS1                                      SpAcc                          Drd1
                    |                                          |                             |
     TCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTT
5301 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5400
     AGCCTTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCATAGAAATATCAGGACAGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAAAA

                                                                                                SpDon
     BpuE1                   SpAcc     Eci1                                                  BspLU|
         |                       |        |                                                       ||
     GTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT
5401 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5500
     CACTACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCAAGGACCGGAAAACGACCGGAAAACGAGTGTACAAGAAA

                                                Nsi1
                                             BfrB1 |
                                                 | |
     CCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT
5501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||| 5548
     GGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTACGTA
```

Found:

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aat2 | **Afe1** | **Afl2** | **Age1** | Ale1 | AlwN1 | ApaL1 | **Ase1** | Avr2 | **BamH1** | Bbs1 | BciV1 | **Bcl1** | BfrB1 |
| BfuA1 | Bgl1 | Bgl2 | **Blp1** | Bmr1 | Bpm1 | BpuE1 | Bsa1 | BsaB1 | **BsaXa** | **BsaXb** | **BseR1** | BseY1 | **Bsg1** |
| Bsm1 | **BspE1** | BspH1 | **BspLU** | BspM1 | BsrB1 | BsrD1 | **BsrG1** | BssS1 | BstAP | BstB1 | Bsu36 | BtgZ1 | **Bts1** |
| **_Chi** | **Cla1** | Dra1 | Dra3 | Drd1 | Eag1 | Ear1 | Eci1 | Ecl2 | Eco57 | **EcoK** | **EcoN1** | **EcoR1** | **Fsp1** |
| **Hind3** | **Hpa1** | **Kas1** | **Mfe1** | Msc1 | Nae1 | **Nar1** | Nco1 | **Nde1** | NgoM4 | **Nhe1** | **Not1** | Nsi1 | **PflF1** |
| PflM1 | polyA | **PshA1** | Psi1 | **Pst1** | Pvu2 | **Rsr2** | Sac1 | **Sal1** | Sap1 | **SexA1** | **Sfi1** | **SnaB1** | SpAcc |
| SpDon | Sph1 | Ssp1 | Stu1 | **Xba1** | **Xho1** | | | | | | | | |

Unique:

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Afe1** | **Afl2** | **Age1** | **Ase1** | **BamH1** | **Bcl1** | **Blp1** | **BsaXa** | **BsaXb** | **BseR1** | **Bsg1** | **BspE1** | **BspLU** | **BsrG1** |
| **Bts1** | **_Chi** | **Cla1** | **EcoK** | **EcoN1** | **EcoR1** | **Fsp1** | **Hind3** | **Hpa1** | **Kas1** | **Mfe1** | **Nar1** | **Nde1** | **Nhe1** |
| **Not1** | **PflF1** | **PshA1** | **Pst1** | **Rsr2** | **Sal1** | **SexA1** | **Sfi1** | **SnaB1** | **Xba1** | **Xho1** | | | |

Not found:

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aar1 | Acc65 | Acl1 | Ahd1 | Apa1 | Asc1 | AsiS1 | Bae1a | Bae1b | BbvC1 | Bcg1a | Bcg1b | BmgB1 | Bpu10 |
| BsiW1 | BsmB1 | BssH2 | BstE2 | BstX1 | BstZ1 | BxatB | BxatL | BxatR | BxatP | EcoRV | FCatB | FCatL | FCatR |
| FCatP | ScFRT | Fse1 | FspA1 | I_Ceu | Kpn1 | loxP | Mlu1 | Nru1 | Pac1 | Pme1 | Pml1 | PspOM | Pvu1 |
| R4atB | R4atL | R4atP | R4atR | Sac2 | SanD1 | Sbf1 | Sca1 | Sgf1 | SgrA1 | Sma1 | Spe1 | Srf1 | Swa1 |
| T3RNA | T7RNA | T7Ter | PISce | Xcm1 | 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 | | | | | | | | | | | | | |