pFusionRed-H2B 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).

FusionRed amino acids are shown in red, H2B 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

                          Hind3
                 Xho1   Sac1 |  EcoR1      Sal1      Kpn1
        BpuE1 Bgl2   | Ecl2 | |BstB1 |     Pst1| Acc65   |
            |    |   |    | | |    | |        ||     |   |
     CCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCCACCATGCCAGAGCCAGCGAAGTCTGCTCCCGCCCCGAAAAAGGGCTC
601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 700
     GGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCAGCTGCCATGGCGGTGGTACGGTCTCGGTCGCTTCAGACGAGGGCGGGGCTTTTTCCCGAG
H2B     >                                                   M  P  E  P  A  K  S  A  P  A  P  K  K  G  S

                                                                                BsrG1  BspM1
                                                                                  |      |
     CAAGAAGGCGGTGACTAAGGCGCAGAAGAAAGGCGGCAAGAAGCGCAAGCGCAGCCGCAAGGAGAGCTATTCCATCTATGTGTACAAGGTTCTGAAGCAG
701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 800
     GTTCTTCCGCCACTGATTCCGCGTCTTCTTTCCGCCGTTCTTCGCGTTCGCGTCGGCGTTCCTCTCGATAAGGTAGATACACATGTTCCAAGACTTCGTC
H2B     >  K  K  A  V  T  K  A  Q  K  K  G  G  K  K  R  K  R  S  R  K  E  S  Y  S  I  Y  V  Y  K  V  L  K  Q

                                 BstX1       EcoR1                    BspM1
                 Eco57           Nco1|   BspH1   |          BtgZ1      Aar1       SpDon
                    |                ||       |   |              |         |           |
     GTCCACCCTGACACCGGCATTTCGTCCAAGGCCATGGGCATCATGAATTCGTTTGTGAACGACATTTTCGAGCGCATCGCAGGTGAGGCTTCCCGCCTGG
801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 900
     CAGGTGGGACTGTGGCCGTAAAGCAGGTTCCGGTACCCGTAGTACTTAAGCAAACACTTGCTGTAAAAGCTCGCGTAGCGTCCACTCCGAAGGGCGGACC
H2B     > V  H  P  D  T  G  I  S  S  K  A  M  G  I  M  N  S  F  V  N  D  I  F  E  R  I  A  G  E  A  S  R  L  A
```

```
                        Afe1
                       Bpm1|                           Eag1                           Msc1       Dra3
                          ||                             |                              |          |
            CGCATTACAACAAGCGCTCGACCATCACCTCCAGGGAGATCCAGACGGCCGTGCGCCTGCTGCTGCCTGGGGAGTTGGCCAAGCACGCCGTGTCCGAGGG
       901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1000
            GCGTAATGTTGTTCGCGAGCTGGTAGTGGAGGTCCCTCTAGGTCTGCCGGCACGCGGACGACGACGGACCCCTCAACCGGTTCGTGCGGCACAGGCTCCC
H2B         >  H  Y  N  K  R  S  T  I  T  S  R  E  I  Q  T  A  V  R  L  L  L  P  G  E  L  A  K  H  A  V  S  E  G

                                        BamH1                SpDon
                                   Bpu10   |               Ale1 |
                                  Afe1 |   |  Age1       Nco1 | |                                BsrG1
                                     | |   |     |          | | |                                   |
            TACTAAGGCCATCACCAAGTACACCAGCGCTAAGGATCCACCGGTCGCCACCATGGTGAGCGAGCTGATTAAGGAGAACATGCCCATGAAGCTGTACATG
      1001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1100
            ATGATTCCGGTAGTGGTTCATGTGGTCGCGATTCCTAGGTGGCCAGCGGTGGTACCACTCGCTCGACTAATTCCTCTTGTACGGGTACTTCGACATGTAC
H2B/FusionRed>  T  K  A  I  T  K  Y  T  S  A  K  D  P  P  V  A  T  M  V  S  E  L  I  K  E  N  M  P  M  K  L  Y  M

                                   ApaL1                     SpAcc
                                      |                         |
            GAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGTCGTCGAGGGCG
      1101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1200
            CTCCCGTGGCACTTGTTGGTGGTGAAGTTCACGTGTAGGCTCCCGCTTCCGTTCGGGATGCTCCCGTGGGTCTGGTACTCTTAGTTCCAGCAGCTCCCGC
FusionRed   > 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  V  V  E  G  G


            GCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAGAACCTTCATCAAGCACCCTCCGGGCATCCCCGACTTCTTTAAGCA
      1201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1300
            CGGGAGAGGGGAAGCGGAAGCTGTAGGACCGATGGTCGAAGTACATGCCGTCGTCTTGGAAGTAGTTCGTGGGAGGCCCGTAGGGGCTGAAGAAATTCGT
FusionRed   >  P  L  P  F  A  F  D  I  L  A  T  S  F  M  Y  G  S  R  T  F  I  K  H  P  P  G  I  P  D  F  F  K  Q


                Bsu36       SpDon                          Bbs1             Bpm1
                    |           |                             |                |
            GTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTC
      1301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1400
            CAGGAAGGGACTCCCGAAGTGTACCCTCTCTCAGTGGTGTATGCTTCTGCCCCCGCACGACTGGCGATGGGTCCTGTGGTCGGAGGTCCTGCCGACGGAG
FusionRed   >  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  G  C  L
```

```
                                                                                                BsmB1
                                        BseY1                                       BseY1    Stu1  |
                                           |                                           |       |  |
            ATCTACAACGTCAAGGTTAGAGGGGTGAACTTCCCAGCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAGACGATGTACC
      1401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1500
            TAGATGTTGCAGTTCCAATCTCCCCACTTGAAGGGTCGGTTGCCGGGACACTACGTCTTCTTTTGTGAGCCGACCCTCCGGAGGTGGCTCTGCTACATGG
FusionRed    > I  Y  N  V  K  V  R  G  V  N  F  P  A  N  G  P  V  M  Q  K  K  T  L  G  W  E  A  S  T  E  T  M  Y  P

                                                                                                            BpuE1
                                Bgl1                      BssS1          Eco57        Bsa1                  BpuE1  |
                                   |                        |              |           |                        |  |
            CCGCTGACGGCGGCCTGGAAGGCGCATGTGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACCTTGAGACCACATACAGATCCAAGAA
      1501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1600
            GGCGACTGCCGCCGGACCTTCCGCGTACACTGTACCGGGACTTCGAGCACCCGCCCCCGGTGGACTAGACGTTGGAACTCTGGTGTATGTCTAGGTTCTT
FusionRed    >   A  D  G  G  L  E  G  A  C  D  M  A  L  K  L  V  G  G  G  H  L  I  C  N  L  E  T  T  Y  R  S  K  K

                                                   SpAcc
                                                   PflM1                  Bsa1       PshA1       BssS1
                                                      |                     |           |           |
            ACCCGCTACGAACCTCAAGATGCCCGGCGTCTACAACGTGGACCACAGACTGGAAAGAATCAAGGAGGCCGACGATGAGACCTACGTCGAGCAGCACGAG
      1601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1700
            TGGGCGATGCTTGGAGTTCTACGGGCCGCAGATGTTGCACCTGGTGTCTGACCTTTCTTAGTTCCTCCGGCTGCTACTCTGGATGCAGCTCGTCGTGCTC
FusionRed    >  P  A  T  N  L  K  M  P  G  V  Y  N  V  D  H  R  L  E  R  I  K  E  A  D  D  E  T  Y  V  E  Q  H  E

                                                      Not1
                                                      Eag1
          Dra3      Msc1                          SpDon    BsrB1|      Xba1   BsaB1
             |         |                             |        ||         |       |
            GTGGCTGTGGCCAGATACTCTACTGGTGGCGCTGGTGATGGAGGTAAATGAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTT
      1701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1800
            CACCGACACCGGTCTATGAGATGACCACCGCGACCACTACCTCCATTTACTCGCCGGCGCTGAGATCTAGTATTAGTCGGTATGGTGTAAACATCTCCAA
FusionRed    > V  A  V  A  R  Y  S  T  G  G  A  G  D  G  G  K  *

                                                                  Bsm1
             SpDon   Dra1                                        Mfe1|        Hpa1       polyA    Psi1
                |      |                                           ||           |           |       |
            TTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTT
      1801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1900
            AATGAACGAAATTTTTTGGAGGGTGTGGAGGGGGACTTGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAATAACGTCGAATATTACCAA
```

```
     polyA                        polyA            Bts1 Bsm1                                Afl2
       |                            |                |    |                                     |
       ACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAAGGCGT
1901   |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2000
       TGTTTATTTCGTTATCGTAGTGTTTAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGTTACATAGAATTCCGCA

       SpDon        Ssp1                                                                          Psi1
           |           |                                                                            |
       AAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAA
2001   |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2100
       TTTAACATTCGCAATTATAAAACAATTTTAAGCGCAATTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTAGCCGTTTTAGGGAATATT

                                                                  BsaXb                 Drd1    BsaXa
                                                                     |                    |        |
       ATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACC
2101   |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2200
       TAGTTTTCTTATCTGGCTCTATCCCAACTCACAACAAGGTCAAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTGCAGTTTCCCGCTTTTTGG

                                BtgZ1
                                Dra3|                                                       SpAcc
                                   ||                                                          |
       GTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCC
2201   |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2300
       CAGATAGTCCCGCTACCGGGTGATGCACTTGGTAGTGGGATTAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGG

                                   Nae1
                                NgoM4 |                                        BsrB1
                                    | |                                          |
       CCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGT
2301   |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2400
       GGGCTAAATCTCGAACTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGACCGTTCACATCGCCA

       CACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGT
2401   |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2500
       GTGCGACGCGCATTGGTGGTGTGGGCGGCGCGAATTACGCGGCGATGTCCCGCGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACA
```

```
                                   BciV1
                                 BspH1  |
      polyA                     BsrB1 |  |                            Ssp1  Ear1        Bsu36
          |                        | |  |                               |     |            |
      TTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTCCTGAGGCGGAAA
2501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2600
      AATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCAGGACTCCGCCTTT

                                                                                      Nsi1
                                                                                    Sph1 |
          Eci1                                                                     BfrB1 |
         Pvu2 |                                      BseY1                     BstAP  | |            SexA1
             | |                                       |                           |  | |                |
      GAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACC
2601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2700
      CTTGGTCGACACCTTACACACAGTCAATCCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGG

                                                      Nsi1
                                                    Sph1 |
                                                   BfrB1 |
                          BseY1                  BstAP  | |                        Eci1
                             |                      |  | |                          |
      AGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCC
2701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2800
      TCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGG

              Eci1                                                                          Sfi1
   Eci1      Bmr1|        Eci1            Nco1                    polyA                    Bgl1
      |         ||          |               |                       |                        |
      CGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATT
2801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2900
      GCGGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGCGGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAGCCGGAGACTCGATAA

                                Avr2
                               Stu1|
          SpDon             BseR1  ||              Cla1              BsaB1                        BspM1
              |                 |  ||                |                 |                            |
      CCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATT
2901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3000
      GGTCTTCATCACTCCTCCGAAAAAACCTCCGGATCCGAAAACGTTTCTAGCTAGTTCTCTGTCCTACTCCTAGCAAAGCGTACTAACTTGTTCTACCTAA
```

```
                Eag1                                        Bmr1
                  |                                           |
      GCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCA
3001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3100
      CGTGCGTCCAAGAGGCCGGCGAACCCACCTCTCCGATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGT

          Nar1
         Kas1|                        Drd1                                                  Msc1
            ||                          |                                                     |
      GCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGG
3101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3200
      CGCGTCCCCGCGGGCCAAGAAAAACAGTTCTGGCTGGACAGGCCACGGGACTTACTTGACGTTCTGCTCCGTCGCGCCGATAGCACCGACCGGTGCTGCC

                Pvu2
            Fsp1   |       PflF1                        Eco57                                    SpDon
                |  |           |                          |                                          |
      GCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCT
3201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3300
      CGCAAGGAACGCGTCGACACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGA

                                                                                  BtgZ1
                            BciV1         BsrD1                              BspM1  |
                                |             |                                  |  |
      TGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGC
3301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3400
      ACGAGGACGGCTCTTTCATAGGTAGTACCGACTACGTTACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCG

                                                   Sap1
                                                   Ear1                           BpuE1
                                                     |                              |
      ATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGC
3401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3500
      TAGCTCGCTCGTGCATGAGCCTACCTTCGGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCG

                Sph1                        Nco1                BtgZ1
                  |                           |                   |
      TCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCAT
3501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3600
      AGTTCCGCTCGTACGGGCTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTA
```

```
              BseY1
             Nae1 |                                          Sap1
         NgoM4 | |        Rsr2         Eci1                 Ear1                  Eco57
             | | |           |            |                    |                     |
     CGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTC
3601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3700
     GCTGACACCGGCCGACCCACACCGCCTGGCGATAGTCCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCGCTTACCCGACTGGCGAAG

                        BtgZ1
  BssS1               BsrB1 |                                          BsrB1          BstB1
     |                    | |                                            |              |
     CTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGAC
3701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3800
     GAGCACGAAATGCCATAGCGGCGAGGGCTAAGCGTCGCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCTTTACTG

                                                                                                Nae1
                         BssS1                                                            NgoM4 |
                         BspM1                       SpAcc                                 Bpm1 |
                             |                          |                                     | |
     CGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGG
3801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3900
     GCTGGTTCGCTGCGGGTTGGACGGTAGTGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAAGGCCCTGCGGCCGACC

                                                Bpm1
                                            SpAcc  |
                                             Avr2  |
                                                |  |
     ATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCG
3901 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4000
     TACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAAGAAGCGGGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTTATGGCCTTCCTTGGGCGC

          polyA        polyA                                                                      Bsa1
              |            |                                                                         |
     CTATGACGGCAATAAAAAGACAGAATAAAACGCACGGTGTTGGGTCGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTGGCACTCTGTCGATACCCC
4001 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4100
     GATACTGCCGTTATTTTTCTGTCTTATTTTGCGTGCCACAACCCAGCAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCGACCGTGAGACAGCTATGGGG
```

```
      ACCGAGACCCCATTGGGGCCAATACGCCCGCGTTTCTTCCTTTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGG
4101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4200
      TGGCTCTGGGGTAACCCCGGTTATGCGGGCGCAAAGAAGGAAAAGGGGTGGGGTGGGGGGTTCAAGCCCACTTCCGGGTCCCGAGCGTCGGTTGCAGCCC

           BstAP
           AlwN1      Bsu36                            Dra1               Dra1
               |          |                               |                  |
      GCGGCAGGCCCTGCCATAGCCTCAGGTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTG
4201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4300
      CGCCGTCCGGGACGGTATCGGAGTCCAATGAGTATATATGAAATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCCTAGATCCACTTCTAGGAAAAAC

        BspH1
            |
      ATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCT
4301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4400
      TATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAACTCTAGGAAAAAAAGA

    BpuE1                                                                                Eco57
       |                                                                                    |
      GCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGC
4401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4500
      CGCGCATTAGACGACGAACGTTTGTTTTTTTGGTGGCGATGGTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAGAAAAAGGCTTCCATTGACCG

                                      SpAcc
                                          |
      TTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGC
4501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4600
      AAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGATCACATCGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGATGTATGGAGCGAGACG

                  AlwN1                     BpuE1
                      |                         |
      TAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTG
4601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4700
      ATTAGGACAATGGTCACCGACGACGGTCACCGCTATTCAGCACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGTCGCCAGCCCGAC
```

```
             ApaL1     BseY1                                          SpAcc
                 |         |                                              |
      AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAA
4701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4800
      TTGCCCCCCAAGCACGTGTGTCGGGTCGAACCTCGCTTGCTGGATGTGGCTTGACTCTATGGATGTCGCACTCGATACTCTTTCGCGGTGCGAAGGGCTT

                            BciV1
                         Eci1   |                    BssS1                                  SpAcc
                            |   |                        |                                      |
      GGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTG
4801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4900
      CCCTCTTTCCGCCTGTCCATAGGCCATTCGCCGTCCCAGCCTTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCATAGAAATATCAGGAC

                         Drd1            BpuE1               SpAcc     Eci1
                            |                |                  |        |
      TCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTT
4901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5000
      AGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAAAACACTACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCAA

                          SpDon                                                 Nsi1
                         BspLU|                                              BfrB1 |
                             ||                                                  | |
      CCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT
5001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*||||| 5085
      GGACCGGAAAACGACCGGAAAACGAGTGTACAAGAAAGGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTACGTA
```

Found:

Aar1	Aat2	**Acc65**	Afe1	**Afl2**	**Age1**	**Ale1**	AlwN1	ApaL1	**Ase1**	Avr2	**BamH1**	**Bbs1**	BciV1
BfrB1	Bgl1	**Bgl2**	Bmr1	Bpm1	**Bpu10**	BpuE1	Bsa1	BsaB1	**BsaXa**	**BsaXb**	**BseR1**	BseY1	Bsm1
BsmB1	BspH1	**BspLU**	BspM1	BsrB1	**BsrD1**	BsrG1	BssS1	BstAP	BstB1	**BstX1**	Bsu36	BtgZ1	**Bts1**
Cla1	Dra1	Dra3	Drd1	Eag1	Ear1	Eci1	**Ecl2**	Eco57	EcoR1	**Fsp1**	**Hind3**	**Hpa1**	**Kas1**
Kpn1	**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:

Aar1	**Acc65**	**Afl2**	**Age1**	**Ale1**	**Ase1**	**BamH1**	**Bbs1**	**Bgl2**	**Bpu10**	**BsaXa**	**BsaXb**	**BseR1**	**BsmB1**
BspLU	**BsrD1**	**BstX1**	**Bts1**	**Cla1**	**Ecl2**	**Fsp1**	**Hind3**	**Hpa1**	**Kas1**	**Kpn1**	**Mfe1**	**Nar1**	**Nde1**
Nhe1	**Not1**	**PflF1**	**PflM1**	**PshA1**	**Pst1**	**Rsr2**	**Sac1**	**Sal1**	**SexA1**	**Sfi1**	**SnaB1**	**Xba1**	**Xho1**

Not found:

Acl1	Ahd1	Apa1	Asc1	AsiS1	Bae1a	Bae1b	BbvC1	Bcg1a	Bcg1b	Bcl1	Blp1	BmgB1	Bsg1
BsiW1	BspE1	BssH2	BstE2	BstZ1	BxatB	BxatL	BxatR	BxatP	_Chi	EcoK	EcoN1	EcoRV	FCatB
FCatL	FCatR	FCatP	ScFRT	Fse1	FspA1	I_Ceu	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													