pFusionRed-endo vector restriction map

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

Unique sites are shown in bold blue. The location given specifies the 3’ end of the cut DNA (the base to the left of the cut site).

FusionRed amino acids are shown in red, c-Myc epitope and Human RhoB GTPase amino acids are shown in green, linker amino acids are shown in black.

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


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


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


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

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


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


                         SpDon
                        Ale1 |
           Age1       Nco1 | |                                 BsrG1                                ApaL1
              |          | | |                                     |                                    |
               CCGGTCGCCACCATGGTGAGCGAGCTGATTAAGGAGAACATGCCCATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCG
          601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 700
               GGCCAGCGGTGGTACCACTCGCTCGACTAATTCCTCTTGTACGGGTACTTCGACATGTACCTCCCGTGGCACTTGTTGGTGGTGAAGTTCACGTGTAGGC
FusionRed    >             M  V  S  E  L  I  K  E  N  M  P  M  K  L  Y  M  E  G  T  V  N  N  H  H  F  K  C  T  S  E


                          SpAcc
                              |
               AGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGTCGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTT
          701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 800
               TCCCGCTTCCGTTCGGGATGCTCCCGTGGGTCTGGTACTCTTAGTTCCAGCAGCTCCCGCCGGGAGAGGGGAAGCGGAAGCTGTAGGACCGATGGTCGAA
FusionRed    >   G  E  G  K  P  Y  E  G  T  Q  T  M  R  I  K  V  V  E  G  G  P  L  P  F  A  F  D  I  L  A  T  S  F

                                                                               Bsu36       SpDon
                                                                                   |           |
               CATGTACGGCAGCAGAACCTTCATCAAGCACCCTCCGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACA
          801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 900
               GTACATGCCGTCGTCTTGGAAGTAGTTCGTGGGAGGCCCGTAGGGGCTGAAGAAATTCGTCAGGAAGGGACTCCCGAAGTGTACCCTCTCTCAGTGGTGT
FusionRed    >  M  Y  G  S  R  T  F  I  K  H  P  P  G  I  P  D  F  F  K  Q  S  F  P  E  G  F  T  W  E  R  V  T  T
```

```
                      Bbs1             Bpm1                                                            BseY1
                         |                |                                                                |
               TACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGGTTAGAGGGGTGAACTTCCCAGCCA
          901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1000
               ATGCTTCTGCCCCCGCACGACTGGCGATGGGTCCTGTGGTCGGAGGTCCTGCCGACGGAGTAGATGTTGCAGTTCCAATCTCCCCACTTGAAGGGTCGGT
FusionRed    > 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  V  R  G  V  N  F  P  A  N
```

```
                                                    BsmB1
                                         BseY1    Stu1  |                                 Bgl1
                                             |       |  |                                    |
               ACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAGACGATGTACCCCGCTGACGGCGGCCTGGAAGGCGCATGTGACATGGCCCT
         1001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1100
               TGCCGGGACACTACGTCTTCTTTTGTGAGCCGACCCTCCGGAGGTGGCTCTGCTACATGGGGCGACTGCCGCCGGACCTTCCGCGTACACTGTACCGGGA
FusionRed    >   G  P  V  M  Q  K  K  T  L  G  W  E  A  S  T  E  T  M  Y  P  A  D  G  G  L  E  G  A  C  D  M  A  L
```

```
                                                                     BpuE1
               BssS1          Eco57        Bsa1                   BpuE1  |
                   |              |           |                       |  |
               GAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACCTTGAGACCACATACAGATCCAAGAAACCCGCTACGAACCTCAAGATGCCCGGCGTCTACAACGTG
         1101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1200
               CTTCGAGCACCCGCCCCCGGTGGACTAGACGTTGGAACTCTGGTGTATGTCTAGGTTCTTTGGGCGATGCTTGGAGTTCTACGGGCCGCAGATGTTGCAC
FusionRed    >  K  L  V  G  G  G  H  L  I  C  N  L  E  T  T  Y  R  S  K  K  P  A  T  N  L  K  M  P  G  V  Y  N  V
```

```
                   SpAcc
                   PflM1                  Bsa1       PshA1       BssS1 Dra3      Msc1
                       |                     |           |           |    |         |
               GACCACAGACTGGAAAGAATCAAGGAGGCCGACGATGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTCTACTGGTGGCGCTGGTGATG
         1201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1300
               CTGGTGTCTGACCTTTCTTAGTTCCTCCGGCTGCTACTCTGGATGCAGCTCGTCGTGCTCCACCGACACCGGTCTATGAGATGACCACCGCGACCACTAC
FusionRed    > D  H  R  L  E  R  I  K  E  A  D  D  E  T  Y  V  E  Q  H  E  V  A  V  A  R  Y  S  T  G  G  A  G  D  G
```

```
                                            Xho1
                                        Bgl2   |
                                      BseR1|   |    Hind3
                                  BpuE1   ||   |   Sac1 |       EcoR1
             SpDon             BspE1  |   ||   | Ecl2 | |   EcoRV   |   Nco1        AlwN1                    BseR1
                 |                 |  |   ||   |    | | |       |   |      |            |                        |
               GAGGTAAAGGTGGAGGAGGTTCCGGATCAGATCTCGAGCTCAAGCTTGATATCGAATTCCCCATGGAGCAGAAGCTGATCTCCGAGGAGGACCTGAACCG
         1301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1400
               CTCCATTTCCACCTCCTCCAAGGCCTAGTCTAGAGCTCGAGTTCGAACTATAGCTTAAGGGGTACCTCGTCTTCGACTAGAGGCTCCTCCTGGACTTGGC
FusionRed/   >   G  K  G  G  G  G  S  G  S  D  L  E  L  K  L  D  I  E  F  P  M  E  Q  K  L  I  S  E  E  D  L  N  R
c-myc


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


                                                                                  Msc1                   Not1
                               EcoRV    BspM1                                 Pvu2   |                   Eag1BsrB1
                                   |        |                                    |   |                      |    |
               TTTGAGAACTATGTGGCAGATATCGAGGTGGATGGAAAGCAGGTAGAGTTGGCTTTGTGGGACACAGCTGGCCAGGAGGACTACGACCGCCTGCGGCCGC
         1501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1600
               AAACTCTTGATACACCGTCTATAGCTCCACCTACCTTTCGTCCATCTCAACCGAAACACCCTGTGTCGACCGGTCCTCCTGATGCTGGCGGACGCCGGCG
GTFase       > F  E  N  Y  V  A  D  I  E  V  D  G  K  Q  V  E  L  A  L  W  D  T  A  G  Q  E  D  Y  D  R  L  R  P  L


                                 Aat2                                                        SanD1
                             Drd1   |                                                       Bpm1 |    SpDon   Xmn1
                                |   |                                                          | |        |      |
               TCTCCTACCCGGACACCGACGTCATTCTCATGTGCTTCTCGGTGGACAGCCCGGACTCGCTGGAGAACATCCCCGAGAAGTGGGTCCCCGAGGTGAAGCA
         1601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1700
```

```
               AGAGGATGGGCCTGTGGCTGCAGTAAGAGTACACGAAGAGCCACCTGTCGGGCCTGAGCGACCTCTTGTAGGGGCTCTTCACCCAGGGGCTCCACTTCGT
GTFase       >   S  Y  P  D  T  D  V  I  L  M  C  F  S  V  D  S  P  D  S  L  E  N  I  P  E  K  W  V  P  E  V  K  H

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

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

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

                   BamH1        Xba1      Bcl1BsaB1                            SpDon   Dra1
                       |           |         |    |                                |      |
               CCTGCCGGGGATCCACCGGATCTAGATAACTGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCT
         2001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2100
               GGACGGCCCCTAGGTGGCCTAGATCTATTGACTAGTATTAGTCGGTATGGTGTAAACATCTCCAAAATGAACGAAATTTTTTGGAGGGTGTGGAGGGGGA

                                    Bsm1
                                   Mfe1|        Hpa1       polyA    Psi1      polyA                        polyA
                                      ||           |           |       |          |                            |
               GAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAAT
         2101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2200
```

```
      CTTGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAATAACGTCGAATATTACCAATGTTTATTTCGTTATCGTAGTGTTTAAAGTGTTTA


               Bts1 Bsm1                                      Mlu1     SpDon        Ssp1
                  |    |                                         |         |           |
      AAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCG
2201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2300
      TTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGTTACATAGAATTGCGCATTTAACATTCGCAATTATAAAACAATTTTAAGCGC

                                                                Psi1
                                                                   |
      TTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTG
2301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2400
      AATTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTAGCCGTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAACTCACAAC

                                                                                            BtgZ1
                         BsaXb                 Drd1    BsaXa                                Dra3|
                             |                    |        |                                   ||
      TTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATC
2401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2500
      AAGGTCAAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTGCAGTTTCCCGCTTTTTGGCAGATAGTCCCGCTACCGGGTGATGCACTTGGTAG


                                                                                              Nae1
                                                      SpAcc                                 NgoM4 |
                                                          |                                     | |
      ACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAAC
2501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2600
      TGGGATTAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGGGGGCTAAATCTCGAACTGCCCCTTTCGGCCGCTTG

                                   BsrB1
                                       |
      GTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTA
2601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2700
      CACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGACCGTTCACATCGCCAGTGCGACGCGCATTGGTGGTGTGGGCGGCGCGAAT
```

```
                                                                       polyA                       BsrB1
                                                                           |                           |
      ATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCT
2701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2800
      TACGCGGCGATGTCCCGCGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGA
    BciV1                                                                   Eci1
 BspH1  |                            Ssp1  Ear1        Bsu36          Pvu2 |
     |  |                               |     |            |                 | |
      CATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTCCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTG
2801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2900
      GTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCAGGACTCCGCCTTTCTTGGTCGACACCTTACACACAGTCAATCCCACAC

                                                Nsi1
                                              Sph1 |
                                             BfrB1 |
                  BseY1                   BstAP  | |            SexA1                     BseY1
                      |                       |  | |                |                         |
      GAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAG
2901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3000
      CTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTC

                    Nsi1
                  Sph1 |
                 BfrB1 |                                                      Eci1
              BstAP  | |                       Eci1                 Eci1     Bmr1|       Eci1
                  |  | |                          |                    |        ||          |
      AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCG
3001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3100
      TTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGC

                                                                                                  Avr2
                                                   Sfi1                                          Stu1|
     Nco1                   polyA                  Bgl1                    SpDon             BseR1  ||
        |                       |                     |                        |                 |  ||
      CCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAG
3101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3200
      GGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAGCCGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATC
```

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                Cla1              BsaB1                       BspM1               Eag1
                   |                  |                           |                  |
      GCTTTTGCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGC
3201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3300
      CGAAAACGTTTCTAGCTAGTTCTCTGTCCTACTCCTAGCAAAGCGTACTAACTTGTTCTACCTAACGTGCGTCCAAGAGGCCGGCGAACCCACCTCTCCG
                                                                            Nar1
                         Bmr1                                              Kas1|
                            |                                                 ||
      TATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGA
3301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3400
      ATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGTCGCGTCCCCGCGGGCCAAGAAAAACAGTTCTGGCT


                                                                                  Pvu2
   Drd1                                                   Msc1                Fsp1   |       PflF1
      |                                                      |                   |   |           |
      CCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACT
3401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3500
      GGACAGGCCACGGGACTTACTTGACGTTCTGCTCCGTCGCGCCGATAGCACCGACCGGTGCTGCCCGCAAGGAACGCGTCGACACGAGCTGCAACAGTGA


                     Eco57                                      SpDon                         BciV1
                         |                                          |                             |
      GAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATG
3501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3600
      CTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAACGAGGACGGCTCTTTCATAGGTAGTACCGACTAC


                                              BtgZ1
        BsrD1                              BspM1  |
            |                                  |  |
      CAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCT
3601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3700
      GTTACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCGTAGCTCGCTCGTGCATGAGCCTACCTTCGGCCAGA


                    Sap1
                    Ear1                           BpuE1                         Sph1
```

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                       |                               |                            |
      TGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTC
3701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3800
      ACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCGAGTTCCGCTCGTACGGGCTGCCGCTCCTAGAGCAG


                                                                               BseY1
                                                                              Nae1 |
        Nco1               BtgZ1                                           NgoM4 | |        Rsr2
           |                   |                                               | | |           |
      GTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATC
3801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3900
      CACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTAGCTGACACCGGCCGACCCACACCGCCTGGCGATAG

                              Sap1                                                        BtgZ1
     Eci1                     Ear1                   Eco57         BssS1                BsrB1 |
        |                        |                       |             |                    | |
      AGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCA
3901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4000
      TCCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCGCTTACCCGACTGGCGAAGGAGCACGAAATGCCATAGCGGCGAGGGCTAAGCGT


                                                                                             BssS1
                                          BsrB1          BstB1                               BspM1
                                              |              |                                   |
      GCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATT
4001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4100
      CGCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCTTTACTGGCTGGTTCGCTGCGGGTTGGACGGTAGTGCTCTAA


                                                             Nae1
                                                          NgoM4 |
                    SpAcc                                  Bpm1 |
                        |                                     | |
      TCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTT
4101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4200
      AGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAGGCCCTGCGGCCGACCTACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAA
```

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                Bpm1
            SpAcc  |
             Avr2  |                                                        polyA        polyA
                |  |                                                            |            |
      CTTCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCAC
4201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4300
      GAAGCGGGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTTATGGCCTTCCTTGGGCGCGATACTGCCGTTATTTTTCTGTCTTATTTTGCGTG
                                                                 Bsa1
                                                                    |
      GGTGTTGGGTCGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTGGCACTCTGTCGATACCCCACCGAGACCCCATTGGGGCCAATACGCCCGCGTTT
4301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4400
      CCACAACCCAGCAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCGACCGTGAGACAGCTATGGGGTGGCTCTGGGGTAACCCCGGTTATGCGGGCGCAAA

                                                                            BstAP
                                                                            AlwN1      Bsu36
                                                                                |          |
      CTTCCTTTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGCCTCAGGTTACTCATA
4401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4500
      GAAGGAAAAGGGGTGGGGTGGGGGGTTCAAGCCCACTTCCGGGTCCCGAGCGTCGGTTGCAGCCCCGCCGTCCGGGACGGTATCGGAGTCCAATGAGTAT


                    Dra1               Dra1                              BspH1
                       |                  |                                  |
      TATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTT
4501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4600
      ATATGAAATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCCTAGATCCACTTCTAGGAAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAA


                                                                     BpuE1
                                                                         |
      TCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCAC
4601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4700
      AGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAACTCTAGGAAAAAAAGACGCGCATTAGACGACGAACGTTTGTTTTTTTGGTG


                                                   Eco57
                                                       |
      CGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCT
```

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4701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4800
      GCGATGGTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAGAAAAAGGCTTCCATTGACCGAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGA


   SpAcc                                                                            AlwN1
       |                                                                                |
      AGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGAT
4801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4900
      TCACATCGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGATGTATGGAGCGAGACGATTAGGACAATGGTCACCGACGACGGTCACCGCTA
          BpuE1                                                            ApaL1     BseY1
              |                                                                    |         |
      AAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGC
4901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5000
      TTCAGCACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGTCGCCAGCCCGACTTGCCCCCCAAGCACGTGTGTCGGGTCGAACCTCG


                                                                                               BciV1
                                       SpAcc                                                Eci1   |
                                           |                                                   |   |
      GAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAG
5001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5100
      CTTGCTGGATGTGGCTTGACTCTATGGATGTCGCACTCGATACTCTTTCGCGGTGCGAAGGGCTTCCCTCTTTCCGCCTGTCCATAGGCCATTCGCCGTC


                    BssS1                                    SpAcc                          Drd1
                        |                                        |                             |
      GGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTT
5101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5200
      CCAGCCTTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCATAGAAATATCAGGACAGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAA


                                                                                              SpDon
        BpuE1               SpAcc     Eci1                                                   BspLU|
            |                   |        |                                                       ||
      TTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCT
5201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5300
      AACACTACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCAAGGACCGGAAAACGACCGGAAAACGAGTGTACAAGA
```

```
                                                   Nsi1
                                                BfrB1 |
                                                    | |
      TTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT
5301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5350
      AAGGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTACGTA
```

Found:

```
    Aat2     Afe1     Age1     Ale1    AlwN1    ApaL1     Ase1     Avr2    BamH1     Bbs1    Bcg1a    Bcg1b    BciV1     Bcl1
   BfrB1     Bgl1     Bgl2     Bmr1     Bpm1    BpuE1     Bsa1    BsaB1    BsaXa    BsaXb    BseR1    BseY1     Bsm1    BsmB1
   BspE1    BspH1    BspLU    BspM1    BsrB1    BsrD1    BsrG1    BssH2    BssS1    BstAP    BstB1    BstX1    Bsu36    BtgZ1
    Bts1     Cla1     Dra1     Dra3     Drd1     Eag1     Ear1     Eci1     Ecl2    Eco57    EcoR1    EcoRV    FspA1     Fsp1
   Hind3     Hpa1     Kas1     Mfe1     Mlu1     Msc1     Nae1     Nar1     Nco1     Nde1    NgoM4     Nhe1     Not1     Nsi1
   PflF1    PflM1    polyA    PshA1     Psi1     Pst1     Pvu2     Rsr2     Sac1    SanD1     Sap1    SexA1     Sfi1    SnaB1
   SpAcc    SpDon     Sph1     Ssp1     Stu1     Xba1     Xcm1     Xho1     Xmn1
```

Unique:

```
    Age1     Ale1     Ase1    BamH1    Bcg1a    Bcg1b     Bcl1     Bgl2    BsaXa    BsaXb    BspE1    BspLU    BsrD1    BsrG1
   BssH2    BstB1    BstX1     Bts1     Cla1     Ecl2    EcoR1    FspA1    Hind3     Hpa1     Kas1     Mfe1     Mlu1     Nar1
    Nde1     Nhe1     Not1    PflF1    PflM1    PshA1     Pst1     Rsr2     Sac1    SanD1    SexA1     Sfi1    SnaB1     Xba1
    Xcm1
```

Not found:

```
    Aar1    Acc65     Acl1     Afl2     Ahd1     Apa1     Asc1    AsiS1    Bae1a    Bae1b    BbvC1     Blp1    BmgB1    Bpu10
    Bsg1    BsiW1    BstE2    BstZ1    BxatB    BxatL    BxatR    BxatP     _Chi     EcoK    EcoN1    FCatB    FCatL    FCatR
   FCatP    ScFRT     Fse1    I_Ceu     Kpn1     loxP     Nru1     Pac1     Pme1     Pml1    PspOM     Pvu1    R4atB    R4atL
```

```
   R4atP   R4atR    Sac2    Sal1    Sbf1    Sca1    Sgf1   SgrA1    Sma1    Spe1    Srf1    Swa1   T3RNA   T7RNA
   T7Ter   PISce

Excluded by site complexity:

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