pFusionRed-CDC42 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, human CDC42 amino acids are shown in green, linker amino acids are shown in black.

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

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

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

                                  BtgZ1 SnaB1                Nco1
                                     |     |                   |
     CATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGA
 301 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 400
     GTACTGGAATACCCTGAAAGGATGAACCGTCATGTAGATGCATAATCAGTAGCGATAATGGTACCACTACGCCAAAACCGTCATGTAGTTACCCGCACCT
                                                Aat2                                                Eci1
                                                  |                                                   |
```

```
            TAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA
        401 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 500
            ATCGCCAAACTGAGTGCCCCTAAAGGTTCAGAGGTGGGGTAACTGCAGTTACCCTCAAACAAAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCAT

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

                        SpDon
                       Ale1 |
         Age1       Nco1 | |                                  BsrG1                                ApaL1
            |          | | |                                     |                                    |
            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

                                      Bgl2
                SpDon              BspE1  BseR1 |                      Bsm1                                  BtgZ1BspLU
                   |                  |      | |                        |                                        |    |
                GAGGTAAAGGTGGAGGAGGTTCCGGACTCAGATCTGGTGGCTCCGCAGGAATGCAGACAATTAAGTGTGTTGTTGTGGGCGATGGTGCTGTTGGTAAAAC
         1301   |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1400
                CTCCATTTCCACCTCCTCCAAGGCCTGAGTCTAGACCACCGAGGCGTCCTTACGTCTGTTAATTCACACAACAACACCCGCTACCACGACAACCATTTTG
FusionRed/    >   G  K  G  G  G  G  S  G  L  R  S  G  G  S  A  G  M  Q  T  I  K  C  V  V  V  G  D  G  A  V  G  K  T
       CDC42

                     EcoRV
                        |
```

```
            ATGTCTCCTGATATCCTACACAACAAACAAATTTCCATCGGAATATGTACCGACTGTTTTTGACAACTATGCAGTCACAGTTATGATTGGTGGAGAACCA
      1401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1500
            TACAGAGGACTATAGGATGTGTTGTTTGTTTAAAGGTAGCCTTATACATGGCTGACAAAAACTGTTGATACGTCAGTGTCAATACTAACCACCTCTTGGT
CDC42          >  C  L  L  I  S  Y  T  T  N  K  F  P  S  E  Y  V  P  T  V  F  D  N  Y  A  V  T  V  M  I  G  G  E  P

                                   Pst1                                                   T7Ter
                                     |                                                        |
            TATACTCTTGGACTTTTTGATACTGCAGGGCAAGAGGATTATGACAGATTACGACCGCTGAGTTATCCACAAACAGATGTATTTCTAGTCTGTTTTTCAG
      1501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1600
            ATATGAGAACCTGAAAAACTATGACGTCCCGTTCTCCTAATACTGTCTAATGCTGGCGACTCAATAGGTGTTTGTCTACATAAAGATCAGACAAAAAGTC
CDC42          > Y  T  L  G  L  F  D  T  A  G  Q  E  D  Y  D  R  L  R  P  L  S  Y  P  Q  T  D  V  F  L  V  C  F  S  V

                    Bsa1                                                  SpDon
                      |                                                     |
            TGGTCTCTCCATCTTCATTTGAAAACGTGAAAGAAAAGTGGGTGCCTGAGATAACTCACCACTGTCCAAAGACTCCTTTCTTGCTTGTTGGGACTCAAAT
      1601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1700
            ACCAGAGAGGTAGAAGTAAACTTTTGCACTTTCTTTTCACCCACGGACTCTATTGAGTGGTGACAGGTTTCTGAGGAAAGAACGAACAACCCTGAGTTTA
CDC42          >   V  S  P  S  S  F  E  N  V  K  E  K  W  V  P  E  I  T  H  H  C  P  K  T  P  F  L  L  V  G  T  Q  I

                                                        Bpm1                  AlwN1
                                                          |                      |
            TGATCTCAGAGATGACCCCTCTACTATTGAGAAACTTGCCAAGAACAAACAGAAGCCTATCACTCCAGAGACTGCTGAAAAGCTGGCCCGTGACCTGAAG
      1701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1800
            ACTAGAGTCTCTACTGGGGAGATGATAACTCTTTGAACGGTTCTTGTTTGTCTTCGGATAGTGAGGTCTCTGACGACTTTTCGACCGGGCACTGGACTTC
CDC42          >  D  L  R  D  D  P  S  T  I  E  K  L  A  K  N  K  Q  K  P  I  T  P  E  T  A  E  K  L  A  R  D  L  K

                                                                                                          BspM1
                                                                                                           Aar1
                                                                                   Bpm1                  Sap1   |
                    Bsg1   Eco57                      Stu1                       Ssp1  |                  Ear1   |
                      |       |                         |                          |   |                     |   |
            GCTGTCAAGTATGTGGAGTGTTCTGCACTTACACAGAAAGGCCTAAAGAATGTATTTGACGAAGCAATATTGGCTGCCCTGGAGCCTCCAGAACCGAAGA
      1801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 1900
            CGACAGTTCATACACCTCACAAGACGTGAATGTGTCTTTCCGGATTTCTTACATAAACTGCTTCGTTATAACCGACGGGACCTCGGAGGTCTTGGCTTCT
CDC42          > A  V  K  Y  V  E  C  S  A  L  T  Q  K  G  L  K  N  V  F  D  E  A  I  L  A  A  L  E  P  P  E  P  K  K

              Bpm1                SpAcc  BamH1        Xba1      Bcl1BsaB1                          SpDon   Dra1
                |                   |      |           |         |    |                               |      |
```

```
            AGAGCCGCAGGTGTGTGCTGCTATGAGGATCCACCGGATCTAGATAACTGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAA
      1901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2000
            TCTCGGCGTCCACACACGACGATACTCCTAGGTGGCCTAGATCTATTGACTAGTATTAGTCGGTATGGTGTAAACATCTCCAAAATGAACGAAATTTTTT
CDC42          >   S  R  R  C  V  L  L  *

                                                Bsm1
                                               Mfe1|        Hpa1       polyA    Psi1      polyA
                                                  ||           |           |       |          |
            CCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGC
      2001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2100
            GGAGGGTGTGGAGGGGGACTTGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAATAACGTCGAATATTACCAATGTTTATTTCGTTATCG

                      polyA            Bts1 Bsm1                                        Mlu1     SpDon
                          |                |    |                                          |         |
            ATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGTAAATTGTAAGCGTTAAT
      2101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2200
            TAGTGTTTAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGTTACATAGAATTGCGCATTTAACATTCGCAATTA

              Ssp1                                                                    Psi1
                 |                                                                      |
            ATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCG
      2201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2300
            TAAAACAATTTTAAGCGCAATTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTAGCCGTTTTAGGGAATATTTAGTTTTCTTATCTGGC

                                                BsaXb                 Drd1    BsaXa
                                                   |                    |        |
            AGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGG
      2301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2400
            TCTATCCCAACTCACAACAAGGTCAAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTGCAGTTTCCCGCTTTTTGGCAGATAGTCCCGCTACC

                BtgZ1
                Dra3|                                                    SpAcc
                   ||                                                       |
            CCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGA
      2401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2500
            GGGTGATGCACTTGGTAGTGGGATTAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGGGGGCTAAATCTCGAACT
                   Nae1
                NgoM4 |                                 BsrB1
```

```
            | |                                         |
     CGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCA
2501 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 2600
     GCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGACCGTTCACATCGCCAGTGCGACGCGCATTGGT

                                                                                                polyA
                                                                                                  |
     CCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACA
2601 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 2700
     GGTGTGGGCGGCGCGAATTACGCGGCGATGTCCCGCGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAAGATTTATGT


                    BciV1
                  BspH1  |                                                                    Eci1
                BsrB1 |  |                             Ssp1  Ear1        Bsu36              Pvu2 |
                    | |  |                                |     |            |                 | |
     TTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTCCTGAGGCGGAAAGAACCAGCTGTGGAATG
2701 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 2800
     AAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCAGGACTCCGCCTTTCTTGGTCGACACCTTAC

                                                                 Nsi1
                                                               Sph1 |
                                                              BfrB1 |
                                BseY1                   BstAP  | |            SexA1
                                    |                      |  | |                |
     TGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCC
2801 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 2900
     ACACAGTCAATCCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTTCAGGGG


                                  Nsi1
                                Sph1 |
                               BfrB1 |                                                          Eci1
       BseY1                  BstAP  | |                        Eci1                  Eci1     Bmr1|
           |                      |  | |                           |                     |         ||
     AGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCC
2901 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 3000
     TCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGG
                                                                                 Sfi1
      Eci1            Nco1                    polyA                  Bgl1                    SpDon
```

```
            |                |                        |                      |                        |
      AGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAG
3001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3100
      TCAAGGCGGGTAAGAGGCGGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAGCCGGAGACTCGATAAGGTCTTCATCACTCCTC

                Avr2
               Stu1|
           BseR1  ||              Cla1              BsaB1                       BspM1               Eag1
               |  ||                 |                 |                           |                   |
      GCTTTTTTGGAGGCCTAGGCTTTTGCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGG
3101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3200
      CGAAAAAACCTCCGGATCCGAAAACGTTTCTAGCTAGTTCTCTGTCCTACTCCTAGCAAAGCGTACTAACTTGTTCTACCTAACGTGCGTCCAAGAGGCC

                                                                                                  Nar1
                                          Bmr1                                                   Kas1|
                                             |                                                      ||
      CCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGT
3201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3300
      GGCGAACCCACCTCTCCGATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGTCGCGTCCCCGCGGGCCA

                                                                                                   Pvu2
                    Drd1                                                  Msc1                Fsp1   |
                       |                                                     |                   |   |
      TCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCT
3301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3400
      AGAAAAACAGTTCTGGCTGGACAGGCCACGGGACTTACTTGACGTTCTGCTCCGTCGCGCCGATAGCACCGACCGGTGCTGCCCGCAAGGAACGCGTCGA

          PflF1                       Eco57                                   SpDon
             |                          |                                        |
      GTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAG
3401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3500
      CACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAACGAGGACGGCTCTTTC

                                                        BtgZ1
            BciV1         BsrD1                       BspM1  |
```

```
                |              |                                    |  |
     TATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAC
3501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3600
     ATAGGTAGTACCGACTACGTTACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCGTAGCTCGCTCGTGCATG

                                   Sap1
                                   Ear1                            BpuE1                        Sph1
                                      |                               |                           |
     TCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCC
3601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3700
     AGCCTACCTTCGGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCGAGTTCCGCTCGTACGGG

                                                                                                  BseY1
                                                                                                 Nae1 |
                         Nco1                BtgZ1                                            NgoM4 | |
                            |                   |                                                | | |
     GACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGG
3701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3800
     CTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTAGCTGACACCGGCCGACC

                                                Sap1
         Rsr2          Eci1                     Ear1                   Eco57         BssS1
            |             |                       |                       |             |
     GTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTAT
3801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3900
     CACACCGCCTGGCGATAGTCCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCGCTTACCCGACTGGCGAAGGAGCACGAAATGCCATA

       BtgZ1
     BsrB1 |                                                BsrB1          BstB1
         | |                                                  |              |
     CGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCA
3901 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4000
     GCGGCGAGGGCTAAGCGTCGCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCTTTACTGGCTGGTTCGCTGCGGGT

                                                                                  Nae1
          BssS1                                                                NgoM4 |
```

```
            BspM1                      SpAcc                                  Bpm1 |
              |                          |                                      | |
      ACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGG
4001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4100
      TGGACGGTAGTGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAAGGCCCTGCGGCCGACCTACTAGGAGGTCGCGCC

                                Bpm1
                            SpAcc  |
                             Avr2  |                                                          polyA
                                |  |                                                            |
      GGATCTCATGCTGGAGTTCTTCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAA
4101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4200
      CCTAGAGTACGACCTCAAGAAGCGGGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTTATGGCCTTCCTTGGGCGCGATACTGCCGTTATTTT

       polyA                                                                  Bsa1
          |                                                                     |
      AGACAGAATAAAACGCACGGTGTTGGGTCGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTGGCACTCTGTCGATACCCCACCGAGACCCCATTGGG
4201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4300
      TCTGTCTTATTTTGCGTGCCACAACCCAGCAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCGACCGTGAGACAGCTATGGGGTGGCTCTGGGGTAACCC

                                                                                                BstAP
                                                                                                AlwN1
                                                                                                   |
      GCCAATACGCCCGCGTTTCTTCCTTTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCAT
4301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4400
      CGGTTATGCGGGCGCAAAGAAGGAAAAGGGGTGGGGTGGGGGGTTCAAGCCCACTTCCGGGTCCCGAGCGTCGGTTGCAGCCCCGCCGTCCGGGACGGTA

     Bsu36                          Dra1              Dra1                            BspH1
        |                             |                 |                                |
      AGCCTCAGGTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAA
4401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4500
      TCGGAGTCCAATGAGTATATATGAAATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCCTAGATCCACTTCTAGGAAAAACTATTAGAGTACTGGTTT

                                                                                      BpuE1
                                                                                        |
```

```
     ATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCT
4501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4600
     TAGGGAATTGCACTCAAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAACTCTAGGAAAAAAAGACGCGCATTAGACGACGA

                                                                    Eco57
                                                                      |
     TGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGAT
4601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4700
     ACGTTTGTTTTTTTGGTGGCGATGGTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAGAAAAAGGCTTCCATTGACCGAAGTCGTCTCGCGTCTA

                   SpAcc
                       |
     ACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTG
4701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4800
     TGGTTTATGACAGGAAGATCACATCGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGATGTATGGAGCGAGACGATTAGGACAATGGTCAC

  AlwN1                    BpuE1                                                                  ApaL1
     |                         |                                                                      |
     GCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCA
4801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4900
     CGACGACGGTCACCGCTATTCAGCACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGTCGCCAGCCCGACTTGCCCCCCAAGCACGT

      BseY1                                            SpAcc
          |                                              |
     CACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAG
4901 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5000
     GTGTCGGGTCGAACCTCGCTTGCTGGATGTGGCTTGACTCTATGGATGTCGCACTCGATACTCTTTCGCGGTGCGAAGGGCTTCCCTCTTTCCGCCTGTC

            BciV1
        Eci1   |                    BssS1                                    SpAcc
```

```
             |   |                        |                                        |
      GTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTC
5001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5100
      CATAGGCCATTCGCCGTCCCAGCCTTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCATAGAAATATCAGGACAGCCCAAAGCGGTGGAG


          Drd1            BpuE1               SpAcc     Eci1
             |               |                   |        |
      TGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGC
5101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5200
      ACTGAACTCGCAGCTAAAAACACTACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCAAGGACCGGAAAACGACCG


            SpDon                                                 Nsi1
           BspLU|                                              BfrB1 |
               ||                                                  | |
      CTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT
5201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||| 5268
      GAAAACGAGTGTACAAGAAAGGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTACGTA
```

Found:

Aar1	Aat2	**Afe1**	**Age1**	**Ale1**	AlwN1	ApaL1	**Ase1**	Avr2	**BamH1**	**Bbs1**	BciV1	**Bcl1**	BfrB1
Bgl1	**Bgl2**	Bmr1	Bpm1	BpuE1	Bsa1	BsaB1	**BsaXa**	**BsaXb**	BseR1	BseY1	**Bsg1**	Bsm1	**BsmB1**
BspE1	BspH1	BspLU	BspM1	BsrB1	**BsrD1**	**BsrG1**	BssS1	BstAP	**BstB1**	Bsu36	BtgZ1	**Bts1**	**Cla1**
Dra1	Dra3	Drd1	**Eag1**	Ear1	Eci1	Eco57	**EcoRV**	**Fsp1**	**Hpa1**	**Kas1**	**Mfe1**	**Mlu1**	Msc1
Nae1	**Nar1**	Nco1	**Nde1**	NgoM4	**Nhe1**	Nsi1	**PflF1**	**PflM1**	polyA	**PshA1**	Psi1	**Pst1**	Pvu2
Rsr2	Sap1	**SexA1**	**Sfi1**	**SnaB1**	SpAcc	SpDon	Sph1	Ssp1	Stu1	**T7Ter**	**Xba1**		

Unique:

Aar1	**Afe1**	**Age1**	**Ale1**	**Ase1**	**BamH1**	**Bbs1**	**Bcl1**	**Bgl2**	**BsaXa**	**BsaXb**	**Bsg1**	**BsmB1**	**BspE1**
BsrD1	**BsrG1**	**BstB1**	**Bts1**	**Cla1**	**Eag1**	**EcoRV**	**Fsp1**	**Hpa1**	**Kas1**	**Mfe1**	**Mlu1**	**Nar1**	**Nde1**
Nhe1	**PflF1**	**PflM1**	**PshA1**	**Pst1**	**Rsr2**	**SexA1**	**Sfi1**	**SnaB1**	**T7Ter**	**Xba1**			

Not found:

Acc65	Acl1	Afl2	Ahd1	Apa1	Asc1	AsiS1	Bae1a	Bae1b	BbvC1	Bcg1a	Bcg1b	Blp1	BmgB1
Bpu10	BsiW1	BssH2	BstE2	BstX1	BstZ1	BxatB	BxatL	BxatR	BxatP	_Chi	Ecl2	EcoK	EcoN1
EcoR1	FCatB	FCatL	FCatR	FCatP	ScFRT	Fse1	FspA1	Hind3	I_Ceu	Kpn1	loxP	Not1	Nru1
Pac1	Pme1	Pml1	PspOM	Pvu1	R4atB	R4atL	R4atP	R4atR	Sac1	Sac2	Sal1	SanD1	Sbf1
Sca1	Sgf1	SgrA1	Sma1	Spe1	Srf1	Swa1	T3RNA	T7RNA	PISce	Xcm1	Xho1	Xmn1	

Excluded by site complexity:

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