pHcRed-Tandem-N2 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 shown in bold blue. The location given specifies the 3’ end of the cut DNA (the base to the left of the cut site).

MCS sequence shown in frame, amino acids coded by MCS and by a linker between two HcRed1 coding sequences (see vector description) shown in black.

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

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

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

                                         SnaB1                Nco1
                                           |                    |
     CATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGA
 301 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 400
     GTACTGGAATACCCTGAAAGGATGAACCGTCATGTAGATGCATAATCAGTAGCGATAATGGTACCACTACGCCAAAACCGTCATGTAGTTACCCGCACCT
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                                                  Aat2                                                  Eci1
                                                    |                                                     |
        TAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA
    401 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 500
        ATCGCCAAACTGAGTGCCCCTAAAGGTTCAGAGGTGGGGTAACTGCAGTTACCCTCAAACAAAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCAT

                                                                                                Nhe1 Afe1
                                                                                                  |    |
        ACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTA
    501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 600
        TGTTGAGGCGGGGTAACTGCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGACCAAATCACTTGGCAGTCTAGGCGATCGCGAT
t-HcRed     >                                                                                   *  R  Y

                                                                BamH1
                                                               Sma1 |
                                                              Apa1| |
                                                         PspOM   || |
                                                         Sac2|   || |
                  Xho1    Hind3  EcoR1      Sal1      Kpn1  ||   || |                  Ale1
        BpuE1 Bgl2   |    Sac1 |BstB1 |     Pst1| Acc65   |  ||   || |    Eag1         Nco1 |  BsrB1   EcoN1
                  |    |        |     |          ||      |  ||   || |         |              | |      |       |
        CCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGCCGGTCGCCACCATGGTGAGCGGCCTGCTG
    601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 700
        GGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCAGCTGCCATGGCGCCCGGGCCCTAGGTGGCCGGCCAGCGGTGGTACCACTCGCCGGACGAC
t-HcRed     >  R  T  Q  I  S  S  S  S  F  E  F  C  S  R  R  Y  R  G  P  G  I  H  R  P  V  A  T  M  V  S  G  L  L

                  Fsp1     BsrG1                                                                   Nae1
                 FspA1 Eco57   |                                                Bcg1b              NgoM4 |
                     |     |   |                                                  |                  | |
        AAGGAGAGTATGCGCATCAAGATGTACATGGAGGGCACCGTGAACGGCCACTACTTCAAGTGCGAGGGCGAGGGCGACGGCAACCCCTTCGCCGGCACCC
    701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 800
        TTCCTCTCATACGCGTAGTTCTACATGTACCTCCCGTGGCACTTGCCGGTGATGAAGTTCACGCTCCCGCTCCCGCTGCCGTTGGGGAAGCGGCCGTGGG
t-HcRed     >  K  E  S  M  R  I  K  M  Y  M  E  G  T  V  N  G  H  Y  F  K  C  E  G  E  G  D  G  N  P  F  A  G  T
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                                 Nar1
            Bcg1a          Pml1      Kas1|                                    Bcg1b                 ApaL1
                |              |         ||                                       |                     |
              AGAGCATGAGAATCCACGTGACCGAGGGCGCCCCCCTGCCCTTCGCCTTCGACATCCTGGCCCCCTGCTGCGAGTACGGCAGCAGGACCTTCGTGCACCA
         801  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 900
              TCTCGTACTCTTAGGTGCACTGGCTCCCGCGGGGGGACGGGAAGCGGAAGCTGTAGGACCGGGGGACGACGCTCATGCCGTCGTCCTGGAAGCACGTGGT
t-HcRed     > Q  S  M  R  I  H  V  T  E  G  A  P  L  P  F  A  F  D  I  L  A  P  C  C  E  Y  G  S  R  T  F  V  H  H

              Bcg1a
                  |
              CACCGCCGAGATCCCCGACTTCTTCAAGCAGAGCTTCCCCGAGGGCTTCACCTGGGAGAGAACCACCACCTACGAGGACGGCGGCATCCTGACCGCCCAC
         901  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1000
              GTGGCGGCTCTAGGGGCTGAAGAAGTTCGTCTCGAAGGGGCTCCCGAAGTGGACCCTCTCTTGGTGGTGGATGCTCCTGCCGCCGTAGGACTGGCGGGTG
t-HcRed     >   T  A  E  I  P  D  F  F  K  Q  S  F  P  E  G  F  T  W  E  R  T  T  T  Y  E  D  G  G  I  L  T  A  H

                            Bgl1             Bpm1       ApaL1                                          BsrB1
                               |                |           |                                              |
              CAGGACACCAGCCTGGAGGGCAACTGCCTGATCTACAAGGTGAAGGTGCACGGCACCAACTTCCCCGCCGACGGCCCCGTGATGAAGAACAAGAGCGGCG
        1001  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1100
              GTCCTGTGGTCGGACCTCCCGTTGACGGACTAGATGTTCCACTTCCACGTGCCGTGGTTGAAGGGGCGGCTGCCGGGGCACTACTTCTTGTTCTCGCCGC
t-HcRed     >  Q  D  T  S  L  E  G  N  C  L  I  Y  K  V  K  V  H  G  T  N  F  P  A  D  G  P  V  M  K  N  K  S  G

                          Ale1
          BseY1   BseY1    Xcm1|                            Eag1                                   Eco57
              |       |       ||                               |                                       |
              GCTGGGAGCCCAGCACCGAGGTGGTGTACCCCGAGAACGGCGTGCTGTGCGGCCGGAACGTGATGGCCCTGAAGGTGGGCGACCGGCACCTGATCTGCCA
        1101  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1200
              CGACCCTCGGGTCGTGGCTCCACCACATGGGGCTCTTGCCGCACGACACGCCGGCCTTGCACTACCGGGACTTCCACCCGCTGGCCGTGGACTAGACGGT
t-HcRed     > G  W  E  P  S  T  E  V  V  Y  P  E  N  G  V  L  C  G  R  N  V  M  A  L  K  V  G  D  R  H  L  I  C  H

                                        BssH2                        Bpm1                   AlwN1
                                           |                            |                      |
              CCACTACACCAGCTACCGGAGCAAGAAGGCCGTGCGCGCCCTGACCATGCCCGGCTTCCACTTCACCGACATCCGGCTCCAGATGCTGCGGAAGAAGAAG
        1201  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1300
              GGTGATGTGGTCGATGGCCTCGTTCTTCCGGCACGCGCGGGACTGGTACGGGCCGAAGGTGAAGTGGCTGTAGGCCGAGGTCTACGACGCCTTCTTCTTC
t-HcRed     >   H  Y  T  S  Y  R  S  K  K  A  V  R  A  L  T  M  P  G  F  H  F  T  D  I  R  L  Q  M  L  R  K  K  K
```

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                                   Sfi1                    BspM1
                Sca1               Bgl1                    BfuA1       Bgl2    Sma1      BsrB1   EcoN1
                  |                  |                        |          |       |          |       |
            GACGAGTACTTCGAGCTGTACGAGGCCAGCGTGGCCCGGTACAGCGACCTGCCCGAGAAGGCCAACAGATCTCCCGGGATGGTGAGCGGCCTGCTGAAGG
      1301  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1400
            CTGCTCATGAAGCTCGACATGCTCCGGTCGCACCGGGCCATGTCGCTGGACGGGCTCTTCCGGTTGTCTAGAGGGCCCTACCACTCGCCGGACGACTTCC
t-HcRed     >  D  E  Y  F  E  L  Y  E  A  S  V  A  R  Y  S  D  L  P  E  K  A  N  R  S  P  G  M  V  S  G  L  L  K

                 Fsp1     BsrG1                                                                  Nae1
                FspA1 Eco57   |                                          Bcg1b                 NgoM4 |     Bcg1a
                    |     |   |                                            |                     | |         |
            AGAGTATGCGCATCAAGATGTACATGGAGGGCACCGTGAACGGCCACTACTTCAAGTGCGAGGGCGAGGGCGACGGCAACCCCTTCGCCGGCACCCAGAG
      1401  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1500
            TCTCATACGCGTAGTTCTACATGTACCTCCCGTGGCACTTGCCGGTGATGAAGTTCACGCTCCCGCTCCCGCTGCCGTTGGGGAAGCGGCCGTGGGTCTC
t-HcRed     > E  S  M  R  I  K  M  Y  M  E  G  T  V  N  G  H  Y  F  K  C  E  G  E  G  D  G  N  P  F  A  G  T  Q  S

                            Nar1
                 Pml1      Kas1|                                      Bcg1b                 ApaL1
                    |         ||                                        |                     |
            CATGAGAATCCACGTGACCGAGGGCGCCCCCCTGCCCTTCGCCTTCGACATCCTGGCCCCCTGCTGCGAGTACGGCAGCAGGACCTTCGTGCACCACACC
      1501  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1600
            GTACTCTTAGGTGCACTGGCTCCCGCGGGGGGACGGGAAGCGGAAGCTGTAGGACCGGGGGACGACGCTCATGCCGTCGTCCTGGAAGCACGTGGTGTGG
t-HcRed     >   M  R  I  H  V  T  E  G  A  P  L  P  F  A  F  D  I  L  A  P  C  C  E  Y  G  S  R  T  F  V  H  H  T

          Bcg1a
            |
            GCCGAGATCCCCGACTTCTTCAAGCAGAGCTTCCCCGAGGGCTTCACCTGGGAGAGAACCACCACCTACGAGGACGGCGGCATCCTGACCGCCCACCAGG
      1601  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1700
            CGGCTCTAGGGGCTGAAGAAGTTCGTCTCGAAGGGGCTCCCGAAGTGGACCCTCTCTTGGTGGTGGATGCTCCTGCCGCCGTAGGACTGGCGGGTGGTCC
t-HcRed     >  A  E  I  P  D  F  F  K  Q  S  F  P  E  G  F  T  W  E  R  T  T  T  Y  E  D  G  G  I  L  T  A  H  Q

                 Bgl1             Bpm1       ApaL1                                                BsrB1 BseY1
                    |                |          |                                                     |     |
            ACACCAGCCTGGAGGGCAACTGCCTGATCTACAAGGTGAAGGTGCACGGCACCAACTTCCCCGCCGACGGCCCCGTGATGAAGAACAAGAGCGGCGGCTG
      1701  |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1800
            TGTGGTCGGACCTCCCGTTGACGGACTAGATGTTCCACTTCCACGTGCCGTGGTTGAAGGGGCGGCTGCCGGGGCACTACTTCTTGTTCTCGCCGCCGAC
t-HcRed     > D  T  S  L  E  G  N  C  L  I  Y  K  V  K  V  H  G  T  N  F  P  A  D  G  P  V  M  K  N  K  S  G  G  W
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```
                    Ale1
          BseY1    Xcm1|                          Eag1                                  Eco57
              |       ||                            |                                      |
          GGAGCCCAGCACCGAGGTGGTGTACCCCGAGAACGGCGTGCTGTGCGGCCGGAACGTGATGGCCCTGAAGGTGGGCGACCGGCACCTGATCTGCCACCAC
     1801 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 1900
          CCTCGGGTCGTGGCTCCACCACATGGGGCTCTTGCCGCACGACACGCCGGCCTTGCACTACCGGGACTTCCACCCGCTGGCCGTGGACTAGACGGTGGTG
t-HcRed     >  E  P  S  T  E  V  V  Y  P  E  N  G  V  L  C  G  R  N  V  M  A  L  K  V  G  D  R  H  L  I  C  H  H

                                 BssH2                        Bpm1                  AlwN1
                                     |                          |                      |
          TACACCAGCTACCGGAGCAAGAAGGCCGTGCGCGCCCTGACCATGCCCGGCTTCCACTTCACCGACATCCGGCTCCAGATGCTGCGGAAGAAGAAGGACG
     1901 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 2000
          ATGTGGTCGATGGCCTCGTTCTTCCGGCACGCGCGGGACTGGTACGGGCCGAAGGTGAAGTGGCTGTAGGCCGAGGTCTACGACGCCTTCTTCTTCCTGC
t-HcRed     >  Y  T  S  Y  R  S  K  K  A  V  R  A  L  T  M  P  G  F  H  F  T  D  I  R  L  Q  M  L  R  K  K  K  D

                                 Sfi1                    BspM1
              Sca1               Bgl1                    BfuA1          Xho1          Xba1   BsaB1
                |                  |                       |             |             |       |
          AGTACTTCGAGCTGTACGAGGCCAGCGTGGCCCGGTACAGCGACCTGCCCGAGAAGGCCAACAGAACTCGAGGCCGCGACTCTAGATCATAATCAGCCAT
     2001 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 2100
          TCATGAAGCTCGACATGCTCCGGTCGCACCGGGCCATGTCGCTGGACGGGCTCTTCCGGTTGTCTTGAGCTCCGGCGCTGAGATCTAGTATTAGTCGGTA
t-HcRed     > E  Y  F  E  L  Y  E  A  S  V  A  R  Y  S  D  L  P  E  K  A  N  R  T  R  G  R  D  S  R  S  *

                                                                                  Bsm1
                                 Dra1                                            Mfe1|        Hpa1
                                    |                                               ||           |
          ACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTA
     2101 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 2200
          TGGTGTAAACATCTCCAAAATGAACGAAATTTTTTGGAGGGTGTGGAGGGGGACTTGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAAT

                Psi1                                                      Bts1 Bsm1
                   |                                                         |    |
          TTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCAT
     2201 |||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||*||||||||||* 2300
          AACGTCGAATATTACCAATGTTTATTTCGTTATCGTAGTGTTTAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTA
```

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            Afl2                      Ssp1
              |                         |
      CAATGTATCTTAAGGCGTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAAT
2301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2400
      GTTACATAGAATTCCGCATTTAACATTCGCAATTATAAAACAATTTTAAGCGCAATTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTA

                 Psi1                                                      BsaXb                 Drd1
                   |                                                         |                     |
      CGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAAC
2401  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2500
      GCCGTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAACTCACAACAAGGTCAAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTG

        BsaXa                                 Dra3
          |                                     |
      GTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATC
2501  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2600
      CAGTTTCCCGCTTTTTGGCAGATAGTCCCGCTACCGGGTGATGCACTTGGTAGTGGGATTAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATTTAG

                                            Nae1
                                          NgoM4 |                                   BsrB1
                                                | |                                   |
      GGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGC
2601  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2700
      CCTTGGGATTTCCCTCGGGGGCTAAATCTCGAACTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCG

      GCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTG
2701  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2800
      CGACCGTTCACATCGCCAGTGCGACGCGCATTGGTGGTGTGGGCGGCGCGAATTACGCGGCGATGTCCCGCGCAGTCCACCGTGAAAAGCCCCTTTACAC

                                                   BciV1
                                                BspH1  |
                                              BsrB1 |  |                            Ssp1  Ear1
                                                  | |  |                              |     |
      CGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGA
2801  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 2900
      GCGCCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCT
```

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                                                                                                                Nsi1
                                                                                                              Sph1 |
                                Eci1                                                                         BfrB1 |
        Bsu36                 Pvu2 |                                        BseY1                     BstAP  | |
            |                    | |                                          |                          |  | |
      AGAGTCCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCAT
2901  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3000
      TCTCAGGACTCCGCCTTTCTTGGTCGACACCTTACACACAGTCAATCCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTA

                                                                                  Nsi1
                                                                                Sph1 |
                                                                               BfrB1 |
                 SexA1                        BseY1                        BstAP  | |                        Eci1
                    |                           |                            |  | |                           |
      CTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGC
3001  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3100
      GAGTTAATCAGTCGTTGGTCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCG

                                Eci1
                      Eci1     Bmr1|       Eci1            Nco1
                         |        ||          |              |
      CCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGC
3101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3200
      GGGATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGCGGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCG

                                                  Avr2
    Sfi1                                         Stu1|
    Bgl1                                      BseR1  ||              Cla1              BsaB1
       |                                         |  ||                 |                 |
      CTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTGGAGGCCTAGGCTTTTGCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCAT
3201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3300
      GAGCCGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATCCGAAAACGTTTCTAGCTAGTTCTCTGTCCTACTCCTAGCAAAGCGTA

              BspM1
              BfuA1                Eag1                                          Bmr1
                  |                  |                                             |
      GATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCC
3301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3400
      CTAACTTGTTCTACCTAACGTGCGTCCAAGAGGCCGGCGAACCCACCTCTCCGATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGG
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                          Nar1
                         Kas1|                        Drd1
                            ||                          |
     GCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTAT
3401 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3500
     CGGCACAAGGCCGACAGTCGCGTCCCCGCGGGCCAAGAAAAACAGTTCTGGCTGGACAGGCCACGGGACTTACTTGACGTTCTGCTCCGTCGCGCCGATA

                                 Pvu2
          Msc1                Fsp1   |       PflF1                        Eco57
             |                   |   |           |                           |
     CGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGA
3501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3600
     GCACCGACCGGTGCTGCCCGCAAGGAACGCGTCGACACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCT

                                                                                                  BspM1
                                                  BciV1         BsrD1                           BfuA1
                                                     |             |                                |
     TCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGAC
3601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3700
     AGAGGACAGTAGAGTGGAACGAGGACGGCTCTTTCATAGGTAGTACCGACTACGTTACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTG

                                                                        Sap1
                                                                        Ear1
                                                                          |
     CACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAG
3701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3800
     GTGGTTCGCTTTGTAGCGTAGCTCGCTCGTGCATGAGCCTACCTTCGGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTC

    BpuE1                         Sph1                        Nco1
       |                            |                           |
     CCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGG
3801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 3900
     GGCTTGACAAGCGGTCCGAGTTCCGCTCGTACGGGCTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACC
```

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                                BseY1
                               Nae1 |                                          Sap1
                            NgoM4 | |        Rsr2         Eci1                Ear1
                                | | |           |            |                     |
     CCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGC
3901 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4000
     GGCGAAAAGACCTAAGTAGCTGACACCGGCCGACCCACACCGCCTGGCGATAGTCCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCG

     Eco57         BssS1                BsrB1                                                  BsrB1
         |             |                    |                                                      |
     GAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGAC
4001 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4100
     CTTACCCGACTGGCGAAGGAGCACGAAATGCCATAGCGGCGAGGGCTAAGCGTCGCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTG

                                          BssS1
                                          BspM1
         BstB1                            BfuA1
             |                                |
     TCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTT
4101 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4200
     AGACCCCAAGCTTTACTGGCTGGTTCGCTGCGGGTTGGACGGTAGTGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAA

             Nae1
          NgoM4 |                                                 Bpm1
           Bpm1 |                                             Avr2  |
              | |                                                 |  |
     TTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAA
4201 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4300
     AAGGCCCTGCGGCCGACCTACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAAGAAGCGGGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTT

     TACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGTGTTGGGTCGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTG
4301 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4400
     ATGGCCTTCCTTGGGCGCGATACTGCCGTTATTTTTCTGTCTTATTTTGCGTGCCACAACCCAGCAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCGAC

                 Bsa1
                    |
     GCACTCTGTCGATACCCCACCGAGACCCCATTGGGGCCAATACGCCCGCGTTTCTTCCTTTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGG
4401 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4500
     CGTGAGACAGCTATGGGGTGGCTCTGGGGTAACCCCGGTTATGCGGGCGCAAAGAAGGAAAAGGGGTGGGGTGGGGGGTTCAAGCCCACTTCCGGGTCCC
```

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                          BstAP
                          AlwN1      Bsu36                            Dra1               Dra1
                              |          |                               |                  |
     CTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGCCTCAGGTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCT
4501 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4600
     GAGCGTCGGTTGCAGCCCCGCCGTCCGGGACGGTATCGGAGTCCAATGAGTATATATGAAATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCCTAGA

                       BspH1
                          |
     AGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTC
4601 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4700
     TCCACTTCTAGGAAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAG

                    BpuE1
                       |
     TTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTT
4701 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4800
     AACTCTAGGAAAAAAAGACGCGCATTAGACGACGAACGTTTGTTTTTTTGGTGGCGATGGTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAGAA

    Eco57
       |
     TTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGC
4801 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 4900
     AAAGGCTTCCATTGACCGAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGATCACATCGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCG

                                   AlwN1                     BpuE1
                                      |                         |
     CTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAA
4901 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5000
     GATGTATGGAGCGAGACGATTAGGACAATGGTCACCGACGACGGTCACCGCTATTCAGCACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATT

                               ApaL1     BseY1
                                  |         |
     GGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAA
5001 |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5100
     CCGCGTCGCCAGCCCGACTTGCCCCCCAAGCACGTGTGTCGGGTCGAACCTCGCTTGCTGGATGTGGCTTGACTCTATGGATGTCGCACTCGATACTCTT
```

```
                                                BciV1
                                             Eci1   |                    BssS1
                                                |   |                        |
      AGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCT
5101  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5200
      TCGCGGTGCGAAGGGCTTCCCTCTTTCCGCCTGTCCATAGGCCATTCGCCGTCCCAGCCTTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGA

                                             Drd1            BpuE1                        Eci1
                                                |                |                            |
      GGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAA
5201  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5300
      CCATAGAAATATCAGGACAGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAAAACACTACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTT

                                              BspLU
                                                |
      CGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATG
5301  |||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||*|||||||||* 5400
      GCGCCGGAAAAATGCCAAGGACCGGAAAACGACCGGAAAACGAGTGTACAAGAAAGGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTAC

    Nsi1
 BfrB1 |
     | |
      CAT
5401  ||| 5403
      GTA
```

Found:

Aat2	**Acc65**	**Afe1**	**Afl2**	Ale1	AlwN1	**Apa1**	ApaL1	**Ase1**	Avr2	**BamH1**	Bcg1a	Bcg1b	BciV1
BfrB1	BfuA1	Bgl1	Bgl2	Bmr1	Bpm1	BpuE1	**Bsa1**	BsaB1	**BsaXa**	**BsaXb**	**BseR1**	BseY1	Bsm1
BspH1	**BspLU**	BspM1	BsrB1	**BsrD1**	BsrG1	BssH2	BssS1	BstAP	BstB1	Bsu36	**Bts1**	**Cla1**	Dra1
Dra3	Drd1	Eag1	Ear1	Eci1	Eco57	EcoN1	**EcoR1**	FspA1	Fsp1	**Hind3**	**Hpa1**	Kas1	**Kpn1**
Mfe1	**Msc1**	Nae1	Nar1	Nco1	**Nde1**	NgoM4	**Nhe1**	Nsi1	**PflF1**	Pml1	Psi1	**PspOM**	**Pst1**
Pvu2	**Rsr2**	**Sac1**	**Sac2**	**Sal1**	Sap1	Sca1	**SexA1**	Sfi1	Sma1	**SnaB1**	Sph1	Ssp1	**Stu1**
Xba1	Xcm1	Xho1											

Unique:

Acc65	**Afe1**	**Afl2**	**Apa1**	**Ase1**	**BamH1**	**Bsa1**	**BsaXa**	**BsaXb**	**BseR1**	**BspLU**	**BsrD1**	**Bts1**	**Cla1**
Dra3	**EcoR1**	**Hind3**	**Hpa1**	**Kpn1**	**Mfe1**	**Msc1**	**Nde1**	**Nhe1**	**PflF1**	**PspOM**	**Pst1**	**Rsr2**	**Sac1**
Sac2	**Sal1**	**SexA1**	**SnaB1**	**Stu1**	**Xba1**								

Not found:

Aar1	Acl1	Age1	Ahd1	Asc1	AsiS1	Bae1a	Bae1b	Bbs1	BbvC1	Bcl1	Blp1	BmgB1	Bpu10
Bsg1	BsiW1	BsmB1	BspE1	BstE2	BstX1	BstZ1	_Chi	EcoK	EcoRV	ScFRT	Fse1	I_Ceu	loxP
Mlu1	Not1	Nru1	Pac1	PflM1	Pme1	PshA1	Pvu1	SanD1	Sbf1	Sgf1	SgrA1	Spe1	Srf1
Swa1	PISce	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													