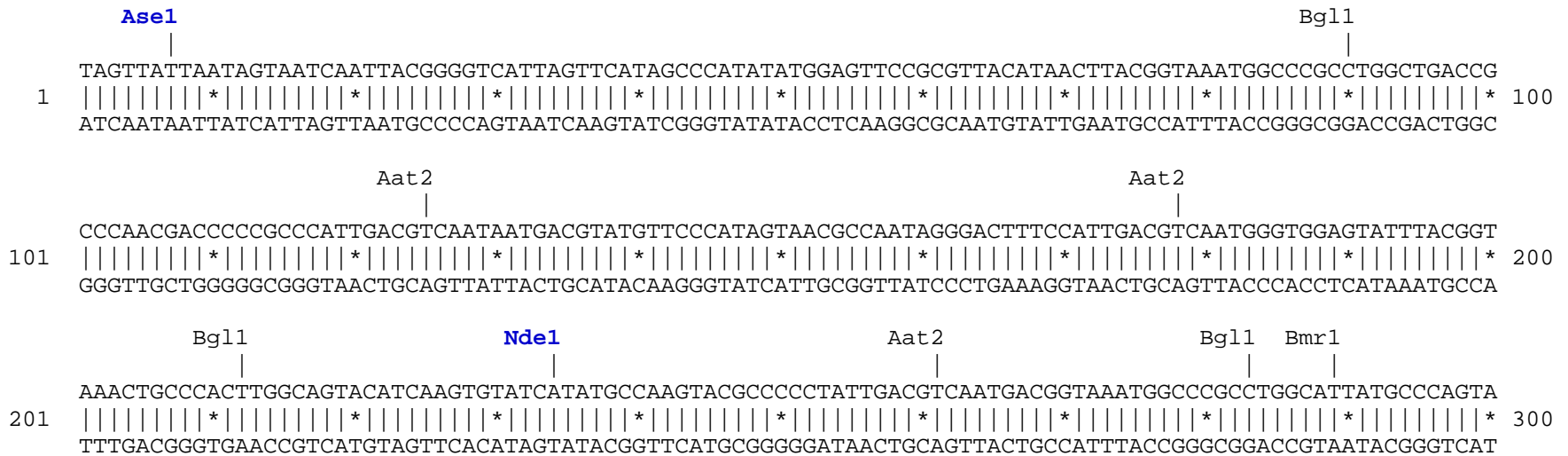


**pHcRed-Tandem-C3 vector** restriction map

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.





```

                                     Bgl1          Bpm1          ApaL1
TACGAGGACGGCGGCATCCTGACCGCCACCAGGACACCAGCCTGGAGGGCAACTGCCTGATCTACAAGGTGAAGGTGCACGGCACCAACTTCCCCGCGG
901  |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1000
ATGCTCCTGCCGCCGTAGGACTGGCGGGTGGTCTGTGGTCCGACCTCCCCGTTGACGGACTAGATGTTCCACTTCCACGTGCCGTGGTTGAAGGGGCGGC
t-HcRed > Y E D G G I L T A H Q D T S L E G N C L I Y K V K V H G T N F P A

                                     BsrB1 BseY1 BseY1 Xcm1 | Eag1
ACGGCCCCGTGATGAAGAACAAGAGCGGCGGGTGGGAGCCCAGCACCGAGGTGGTGTACCCCCGAGAACGGCGTGCTGTGCGGCCGGAACGTGATGGCCCT
1001 |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1100
TGCCGGGGCACTACTTCTTGTTCGCGCCGACCCCTCGGGTTCGTGGCTCCACCACATGGGGCTCTTGCCGCACGACACGCCGGCCTTGCACTACCGGGA
t-HcRed > D G P V M K N K S G G W E P S T E V V Y P E N G V L C G R N V M A L

                                     Eco57          BssH2          Bpm1
GAAGGTGGGCGACCGGCACCTGATCTGCCACCACTACACCAGCTACCGGAGCAAGAAGGCCGTGCGCGCCCTGACCATGCCCGGCTTCCACTTCACCGAC
1101 |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1200
CTTCCACCCGCTGGCCGTGGACTAGACGGTGGTGTGATGTGGTTCGATGGCCTCGTTCTTCCGGCACGCGCGGGACTGGTACGGGCCGAAGGTGAAGTGGCTG
t-HcRed > K V G D R H L I C H H Y T S Y R S K K A V R A L T M P G F H F T D

                                     AlwN1          Scal          Sfi1          BspM1          BfuA1          Bgl2
ATCCGGCTCCAGATGCTGCGGAAGAAGAAGGACGAGTACTTCGAGCTGTACGAGGCCAGCGTGGCCCCGGTACAGCGACCTGCCCGAGAAGGCCAACAGAT
1201 |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1300
TAGGCCGAGGTCTACGACGCCTTCTTCTTCTCCTGCTCATGAAGCTCGACATGCTCCGGTTCGCACCGGGCCATGTCGCTGGACGGGCTCTTCCGGTTGTCTA
t-HcRed > I R L Q M L R K K K D E Y F E L Y E A S V A R Y S D L P E K A N R

                                     Sma1          BsrB1          EcoN1          Fsp1          BsrG1          Bcg1b
CTCCCGGATGGTGAGCGGCCTGCTGAAGGAGAGTATGCGCATCAAGATGTACATGGAGGGCACCGTGAACGGCCACTACTTCAAGTGCAGGGGCGAGGG
1301 |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1400
GAGGGCCCTACCACTCGCCGGACGACTTCTCTCATAACGCGTAGTTCTACATGTACCTCCCGTGGCACTTGCCGGTGTGAAAGTTCACGCTCCCGCTCCC
t-HcRed > S P G M V S G L L 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

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                NaeI      NgoM4      BcgIa      PmlI      KasI      NarI      BcgIb
                |      |      |      |      |      |      |
1401  CGACGGCAACCCCTTCGCCGGCAGCCAGAGCATGAGAATCCACGTGACCGAGGGCGCCCCCTGCCCTTCGCCTTCGACATCCTGGCCCCCTGCTGCGAG
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1500
    GCTGCCGTTGGGGAAGCGGCCGTGGGTCTCGTACTCTTAGGTGCACTGGCTCCCCGCGGGGGACGGGAAGCGGAAGCTGTAGGACCGGGGGACGACGCTC
t-HcRed >  D G N P F A G T 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

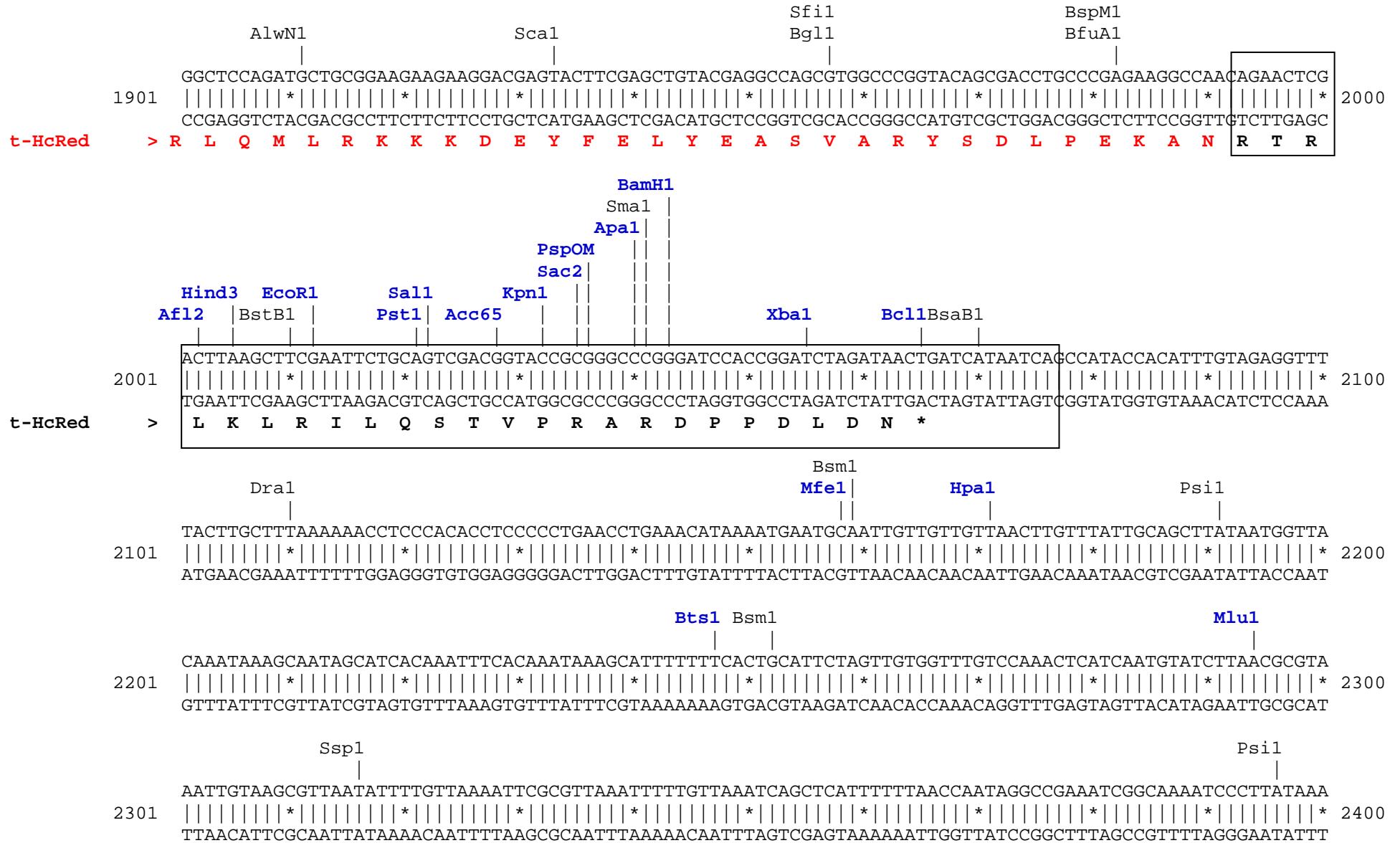
                ApaI      BcgIa
                |      |
1501  TACGGCAGCAGGACCTTCGTGCACCACACCGCCGAGATCCCCGACTTCTTCAAGCAGAGCTTCCCCGAGGGCTTCACTGGGAGAGAACCACCACCTACG
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1600
    ATGCCGTCGTCTGGAAGCACGTGGTGTGGCGGCTCTAGGGGCTGAAGAAGTTCGTCTCGAAGGGGCTCCCGAAGTGGACCCTCTCTTGGTGGTGGATGC
t-HcRed >  Y G S R T F V H H T A E I P D F F K Q S F P E G F T W E R T T T Y

                BglI      BpmI      ApaI
                |      |      |
1601  AGGACGGCGGCATCCTGACCGCCCACCAGGACACCAGCCTGGAGGGCAACTGCCTGATCTACAAGGTGAAGGTGCACGGCACCAACTTCCCCGCGACGG
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1700
    TCCTGCCGCCGTAGGACTGGCGGGTGGTCTGTGGTTCGGACCTCCCCGTTGACGGACTAGATGTTCCACTTCCACGTGCCGTGGTTGAAGGGGGCGGCTGCC
t-HcRed >  E D G G I L T A H 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

                BsrB1  BseY1  BseY1  XcmI      AleI      EagI
                |      |      |      |      |      |
1701  CCCCCTGATGAAGAACAAGAGCGGCGGCTGGGAGCCCAGCACCGAGGTGGTGTACCCCCGAGAACGGCGTGCTGTGCGGCCGGAACGTGATGGCCCTGAAG
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1800
    GGGGCACTACTTCTTGTTCGCGCCGACCCCTCGGGTTCGTGGCTCCACCACATGGGGCTCTTGCCGCACGACACGCCGGCCTTGCACTACCGGGACTTC
t-HcRed >  P V M K N K S G 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

                Eco57      BssH2      BpmI
                |      |      |
1801  GTGGGCGACCGGCACCTGATCTGCCACCACTACACCAGCTACCGGAGCAAGAAGGCCGTGCGCGCCCTGACCATGCCCGGCTTCACTTACCGACATCC
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 1900
    CACCCGCTGGCCGTGGACTAGACGGTGGTGTGATGTGGTTCGATGGCCTCGTTCCTCCGGCACGCGCGGGACTGGTACGGGCCGAAGGTGAAGTGGCTGTAGG
t-HcRed >  V G D R H L I C H H Y T S Y R S K K A V R A L T M P G F H F T D I

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TCAAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCG 2500  
 |||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*||  
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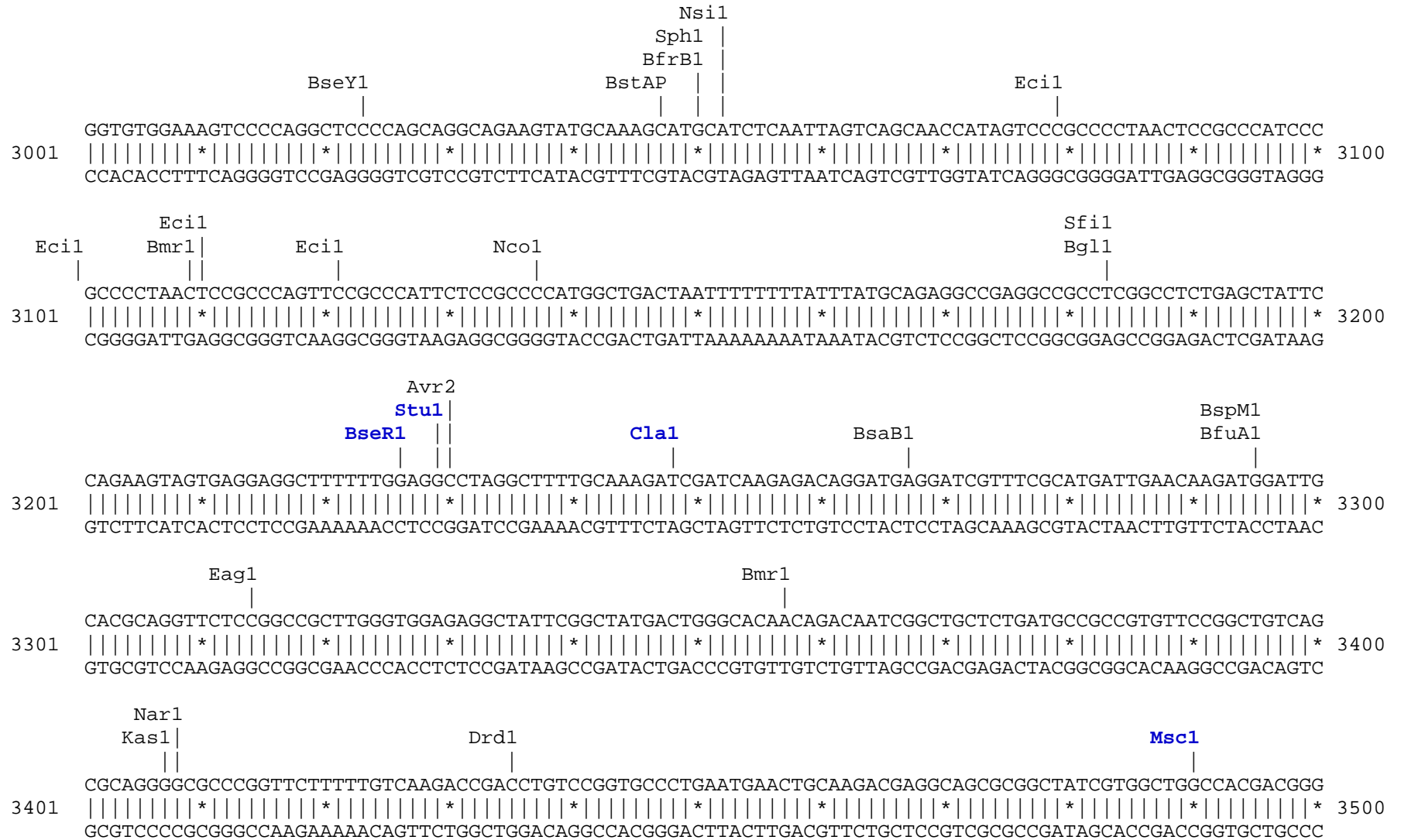
TCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCCTAAAGGGAGCCC 2600  
 |||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*||  
 AGATAGTCCCGCTACCGGGTGATGCACCTGGTAGTGGGATTAGTTCAAAAAACCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGGG

CCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTC 2700  
 |||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*||  
 GGCTAAATCTCGAACTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGACCGTTCACATCGCCAG

ACCTGCGCGTAACCACCACACCCCGCGCTTAATGCGCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTT 2800  
 |||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*||  
 TGCGACGCGCATTGGTGGTGTGGGCGGCGGAATTACGCGCGATGTCCC GCGCAGTCCACCGTAAAAGCCCTTTACACGCGCCTTGGGGATAAAACAA

TATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTCCTGAGGCGGAAAG 2900  
 |||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*||  
 ATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCTTCTCAGGACTCCGCTTTTC

AACCGCTGTGGAATGTGTGTCAGTTAGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCA 3000  
 |||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*|||||\*||  
 TTGGTCGACACCTTACACACAGTCAATCCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGT



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          Pvu2
    Fsp1  |      PflF1      Eco57
    |     |      |          |
3501 CGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTT
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3600
    GCAAGGAACGCGTCGACACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAA

          BciV1      BsrD1      BspM1
          |          |          |
3601 GCTCCTGCCGAGAAAAGTATCCATCATGGCTGATGCAATGCGGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCA
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3700
    CGAGGACGGCTCTTTCATAGGTAGTACCGACTACGTTACGCCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTTCGCTTTGTAGCGT

          Sap1
          Ear1      BpuE1
          |          |          |
3701 TCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTGCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGTGTTCGCCAGGCT
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3800
    AGCTCGCTCGTGCATGAGCCTACCTTCGGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTTCGGCTTGACAAGCGGTCCGA

          Sph1      Nco1
          |          |
3801 CAAGGCGAGCATGCCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTTCATC
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3900
    GTTCCGCTCGTACGGGCTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTAG

          BseY1
          Nae1  |      Rsr2      Eci1      Sap1      Eco57
          NgoM4 |      |          |          Ear1      |
3901 GACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCC
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 4000
    CTGACACCGGCCGACCCACACCGCCTGGCGATAGTCCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACC GCCGCTTACCCGACTGGCGAAGG

    BssS1      BsrB1      BsrB1      BstB1
    |          |          |          |
4001 TCGTGCTTTACGGTATCGCCGCTCCCGATTTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACC
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 4100
    AGCACGAAATGCCATAGCGGCGAGGGCTAAGCGTCGCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCTTTACTGG

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          BssS1      BspM1      BfuA1      NaeI      NgoM4      Bpm1
          |          |          |          |          |          |
4101  GACCAAGCGACGCCAACCTGCCATCACGAGATTTTCGATTCCACCGCCGCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGA
      |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|| 4200
      CTGGTTCGCTGCGGGTTGGACGGTAGTGCTCTAAAGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAAAGGCCCTGCGGCCGACCT

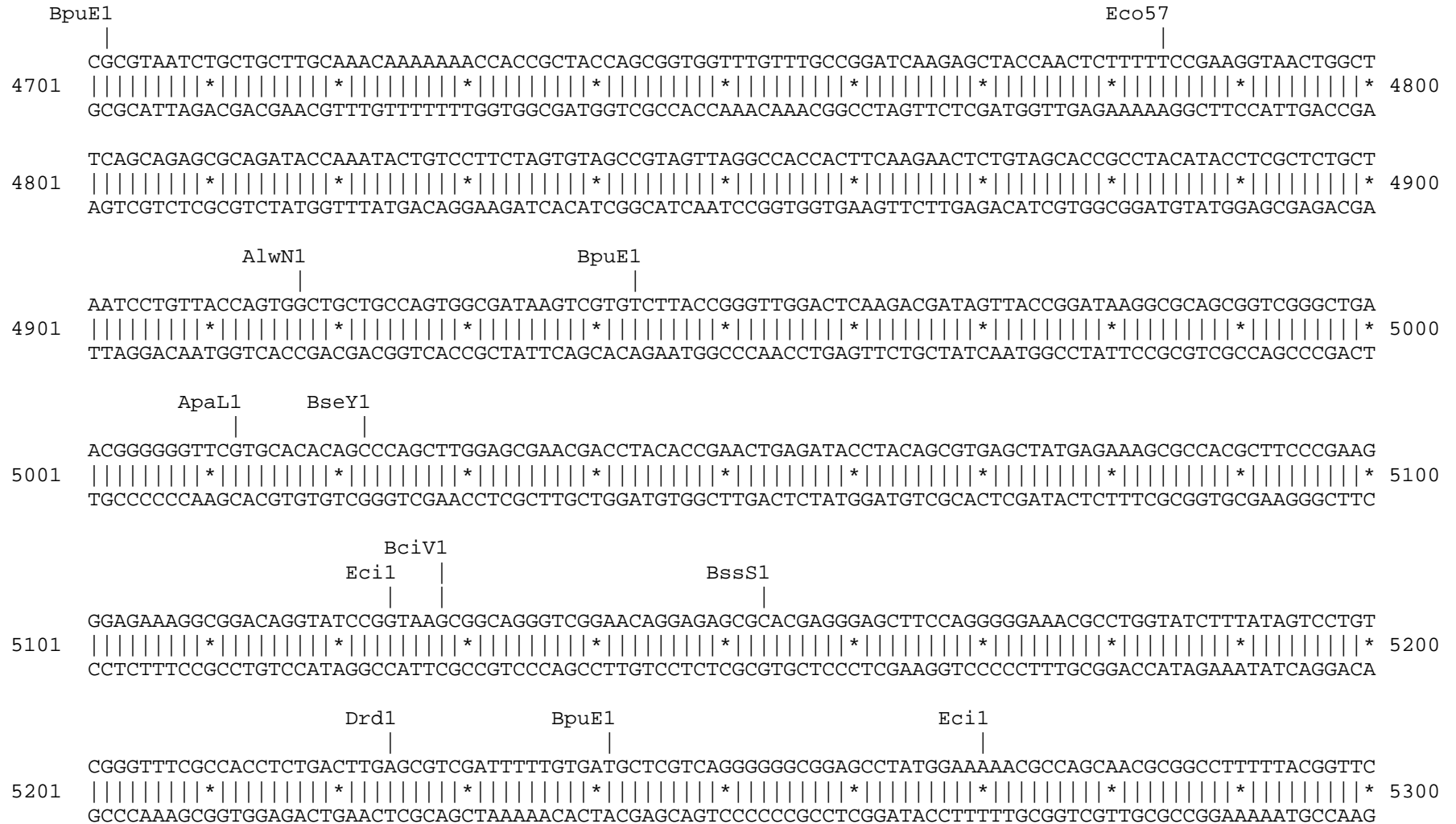
                          Bpm1      Avr2      |
                          |          |          |
4201  TGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGC
      |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|| 4300
      ACTAGGAGGTCGCGCCCTAGAGTACGACCTCAAGAAGCGGGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTTATGGCCTTCCTTGGGCGCG

                                                    BsaI
                                                    |
4301  TATGACGGCAATAAAAAAGACAGAATAAAAACGCACGGTGTGGGTTCGTTTGTTCATAAACCGGGGTTTCGGTCCCAGGGCTGGCACTCTGTGATAACCCCA
      |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|| 4400
      ATACTGCCGTTATTTTTCTGTCTTATTTTTCGCTGCCACAACCCAGCAAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCAGCCGTGAGACAGCTATGGGGT

4401  CCGAGACCCCATTTGGGGCCAATACGCCCGGTTTTCTTCCTTTTCCCACCCCACCCCAAGTTTCGGGTGAAGGCCAGGGCTCGCAGCCAACGTTCGGGG
      |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|| 4500
      GGCTCTGGGGTAACCCCGTTATGCGGGCGCAAAGAAGGAAAAGGGGTGGGGTTCAAGCCCACTTCCGGGTCCCAGCGTCGGTTGCAGCCCC

          BstAP      AlwN1      Bsu36      DraI      DraI
          |          |          |          |          |
4501  CGGCAGGCCCTGCCATAGCCTCAGGTTACTCATATATACTTTAGATTGATTTAAAACCTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGA
      |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|| 4600
      GCCGTCCGGGACGGTATCGGAGTCCAATGAGTATATATGAAATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCTAGATCCACTTCTAGGAAAAAAT

          BspH1
          |
4601  TAATCTCATGACCAAAATCCCTTAAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTG
      |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|| 4700
      ATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCTAGAAAGAACTCTAGGAAAAAAGAC
    
```



Nsi1

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          BspLU                                     BfrB1 |
          |                                         | |
5301 CTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT
      |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*
      GACCGGAAAACGACCGGAAAACGAGTGTACAAGAAAGGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTACGTA 5384
  
```

Found:

Aat2	<b>Acc65</b>	<b>Afe1</b>	<b>Afl2</b>	<b>Age1</b>	Ale1	AlwN1	<b>Apa1</b>	ApaL1	<b>Ase1</b>	Avr2	<b>BamH1</b>	Bcg1a	Bcg1b
BciV1	<b>Bcl1</b>	BfrB1	BfuA1	Bgl1	<b>Bgl2</b>	Bmr1	Bpm1	BpuE1	<b>Bsa1</b>	BsaB1	<b>BsaXa</b>	<b>BsaXb</b>	<b>BseR1</b>
BseY1	Bsm1	BspH1	<b>BspLU</b>	BspM1	BsrB1	<b>BsrD1</b>	BsrG1	BssH2	BssS1	BstAP	BstB1	Bsu36	<b>Bts1</b>
<b>Clal</b>	Dra1	<b>Dra3</b>	Drd1	Eag1	Ear1	Eci1	Eco57	EcoN1	<b>EcoR1</b>	FspA1	Fsp1	<b>Hind3</b>	<b>Hpa1</b>
Kas1	<b>Kpn1</b>	<b>Mfe1</b>	<b>Mlu1</b>	<b>Msc1</b>	Nae1	Nar1	Nco1	<b>Nde1</b>	NgoM4	<b>Nhe1</b>	Nsi1	<b>PflF1</b>	Pml1
Psi1	<b>PspOM</b>	<b>Pst1</b>	Pvu2	<b>Rsr2</b>	<b>Sac2</b>	<b>Sall</b>	Sap1	Sca1	<b>SexA1</b>	Sfi1	Sma1	<b>SnaB1</b>	Sph1
Ssp1	<b>Stu1</b>	<b>Xba1</b>	Xcm1										

Unique:

Acc65	Afe1	Afl2	Age1	Apa1	Ase1	BamH1	Bcl1	Bgl2	Bsa1	BsaXa	BsaXb	BseR1	BspLU
BsrD1	Bts1	Cla1	Dra3	EcoR1	Hind3	Hpa1	Kpn1	Mfe1	Mlu1	Msc1	Nde1	Nhe1	PflF1
PspOM	Pst1	Rsr2	Sac2	Sal1	SexA1	SnaB1	Stu1	Xba1					

Not found:

Aar1	Ac11	Ahd1	Asc1	AsiS1	Baela	Baelb	Bbs1	BbvC1	Blp1	BmgB1	Bpu10	Bsg1	BsiW1
BsmB1	BspE1	BstE2	BstX1	BstZ1	_Chi	EcoK	EcoRV	ScFRT	Fse1	I_Ceu	loxP	Not1	Nru1
Pac1	PflM1	Pme1	PshA1	Pvu1	Sac1	SanD1	Sbf1	Sgf1	SgrA1	Spe1	Srf1	Swal	PISce
Xho1	Xmn1												

Excluded by site complexity:

Acc1	Ac11	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	Tfil	Tse1	Tsp45	Tsp50
TspR1													