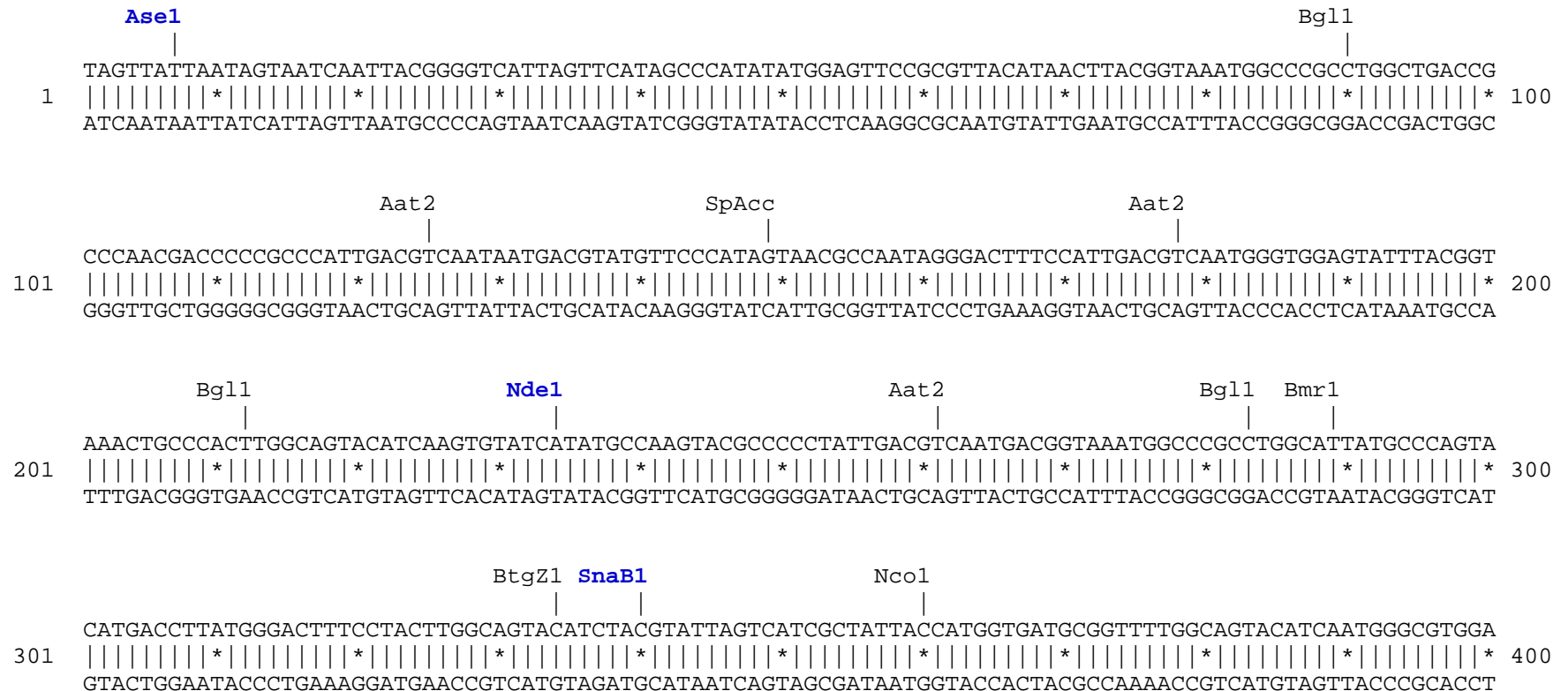


### pTagRFP-Cx32 vector restriction map

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

Unique sites are shown in bold blue. The location given specifies the 3' end of the cut DNA (the base to the left of the cut site). TagRFP amino acids are shown in red, Connexin 32 amino acids are shown in green, linker amino acids are shown in black.





```

          SpAcc
          Pst1|
                      BseY1
                      BseR1|
                        BssS1|
                        Msc1|
                          Pml1
                          BstX1|
                          SpDon
101  TTCCCCATCTCCCATGTGCGGCTGTGGTCCCTGCAGCTCATCCTAGTTTCCACCCCAGCTCTCTCGTGGCCATGCACGTGGCTCACCAGCAACACATAG
    |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*
    AAGGGGTAGAGGGTACACGCCGACACCAGGGACGTTCGAGTAGGATCAAAGGTGGGGTTCGAGAGGAGCACCAGTACGTGCACCGAGTGGTTCGTTGTGTATC
Cx32 > F P I S H V R L W S L Q L I L V S T P A L L V A M H V A H Q Q H I E

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```

                                SpDon
                                Ear1|
                                  EcoN1|
                              Nco1|
                      SanD1|
                      BpuE1|
                SpAcc|
                BseR1|
                Bpm1|
                Ale1|
                PshA1|
101  AGAAGAAAATGCTACGGCTTGAGGGCCATGGGGACCCCCTACACCTGGAGGAGGTGAAGAGGCACAAGGTCCACATCTCAGGGACACTGTGGTGGACCTA
    |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*
    TCTTCTTTTACGATGCCGAACTCCCGGTACCCCTGGGGGATGTGGACCTCCTCCTCTCCGTGTTCCAGGTGTAGAGTCCCTGTGACACCACCTGGAT
Cx32 > K K M L R L E G H G D P L H L E E V K R H K V H I S G T L W W T Y

```

```

                                Bbs1
                                Nco1|
                                Xcm1|
1101  TGTCATCAGCGTGGTGTTCGGCTGTTGTTTGAGGCCGTCTTCATGTATGTCTTTTATCTGCTCTACCCTGGCTATGCCATGGTGGCGGCTGGTCAAGTGC
    |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*
    ACAGTAGTCGCACCACAAGGCCGACAACAAACTCCGGCAGAAGTACATACAGAAAATAGACGAGATGGGACCGATACGGTACCACGCCGACAGTTTACG
Cx32 > V I S V V F R L L F E A V F M Y V F Y L L Y P G Y A M V R L V K C

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```

          Aat2
          BstX1
                      Bbs1
                      Bbs1
                      Nhe1
1201  GACGTCTACCCCTGCCCCAACACAGTGGACTGCTTCGTGTCCCGCCCCACCGAGAAAACCGTCTTCACCGTCTTCATGCTAGCTGCCTCTGGCATCTGCA
    |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*
    CTGCAGATGGGGACGGGGTTGTGTCACTGACGAAGCACAGGGCGGGGTGGCTCTTTTTGGCAGAAGTGGCAGAAGTACGATCGACGGAGACCGTAGACGT
Cx32 > D V Y P C P N T V D C F V S R P T E K T V F T V F M L A A S G I C I

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```

                                BseY1
                                BsrB1
1301  TCATCTCAATGTGGCCGAGGTGGTGTACCTCATCATCCGGGCCTGTGCCCGCCGAGCCCAGCGCCGCTCCAATCCACCTTCCCGLAAGGGCTCGGGCTT
    |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*
    AGTAGGAGTTACACCGGCTCCACCACATGGAGTAGTAGGCCCAGACACGGGGCGGCTCGGGTTCGGCGGAGTTAGGTGGAAGGGCGTTCCCAGCCCGAA
Cx32 > I L N V A E V V Y L I I R A C A R R A Q R R S N P P S R K G S G F

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```

                SpDon
                |
CGGCCACCGCCTCTCACCTGAATACAAGCAGAATGAGATCAACAAGCTGCTGAGTGAGCAGGATGGCTCCCTGAAAGACATACTGCGCCGCAGCCCTGGC
1401 |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||* 1500
GCCGGTGGCGGAGAGTGGACTTATGTTTCGTCTTACTCTAGTTGTTTCGACGACTCACTCGTCTTACCAGGGACTTTCTGTATGACGCGGCGTCTGGGACCG
Cx32 > G H R L S P E Y K Q N E I N K L L S E Q D G S L K D I L R R S P G

                BseY1      SpAcc
                |          |
                BamH1    AgeI
                |          |
                AleI      SapI
                |          |
                NcoI      EarI
ACCGGGGCTGGGCTGGCTGAAAAGAGCGACCGCTGCTCGGCCTGCGCGGATCCACCGGTGCGCCACCATGGTGTCTAAGGGCGAAGAGCTGATTAAGGAGA
1501 |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||* 1600
TGGCCCCGACCCGACCGACTTTTCTCGCTGGCGACGAGCCGGACGCGCCTAGGTGGCCAGCGGTGGTACCACAGATTCCCCTTCTCGACTAATTCCTCT
Cx32/TagRFP > T G A G L A E K S D R C S A C A D P P V A T M V S K G E E L I K E N

                BsrG1
                |
                ApaL1
                |
                SpAcc
                |
ACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCCTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCAT
1601 |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||* 1700
TGTACGTGTACTTTCGACATGTACCTCCCCTGGCACTTGTGGTGGTGAAGTTCACGTGTAGGCTCCCCTTCCGTTTCGGGATGCTCCCCTGGGTCTGGTA
TagRFP > M H M K L Y M E G T V N N H H F K C T S E G E G K P Y E G T Q T M

GAGAATCAAGGTGGTTCGAGGGCGGCCCTCTCCCCTTCGCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAGAACCTTCATCAACCACACCCAG
1701 |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||* 1800
CTCTTAGTTCCACCAGCTCCCCTCCGGAGAGGGGAAGCGGAAGCTGTAGGACCGATGGTTCGAAGTACATGCCGTTCGTTGGAAGTAGTTGGTGTGGGTC
TagRFP > R I K V V E G G P L P F A F D I L A T S F M Y G S R T F I N H T Q

                Bsu36      SpDon
                |          |
                Bbs1      Bpm1
                |          |
GGCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCAACACATACGAAGACGGGGGCGTGTGACCGCTACCCAGGACA
1801 |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||* 1900
CCGTAGGGGCTGAAGAAATTCGTTCAGGAAGGGACTCCCAGAGTGTACCCTCTCTCAGTGGTGTATGCTTCTGCCCCCGCACGACTGGCGATGGGTCTGT
TagRFP > G I P D F F K Q S F P E G F T W E R V T T Y E D G G V L T A T Q D T

                BseY1
                |
CCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGA
1901 |||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||*||||||* 2000
GGTCGGAGGTCTTGCCGACGGAGTAGATGTTGAGTTCTAGTCTCCCCACTTGAAGGGTAGGTTGCCGGGACACTACGTCTTCTTTTGTGAGCCGACCT
TagRFP > S L Q D G C L I Y N V K I R G V N F P S N G P V M Q K K T L G W E

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                Ecil
          Ear1   Bsu36           Pvu2 |           BseY1
          |     |               | |           |
3101 TTGAAAAAGGAAGAGTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGC
   |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3200
   AACTTTTCTCTCAGGACTCCGCTTTCTTGGTCGACACCTTACACACAGTCAATCCCACACCTTTCAGGGGTCCGAGGGGTTCGTCCGTCTTCATACG

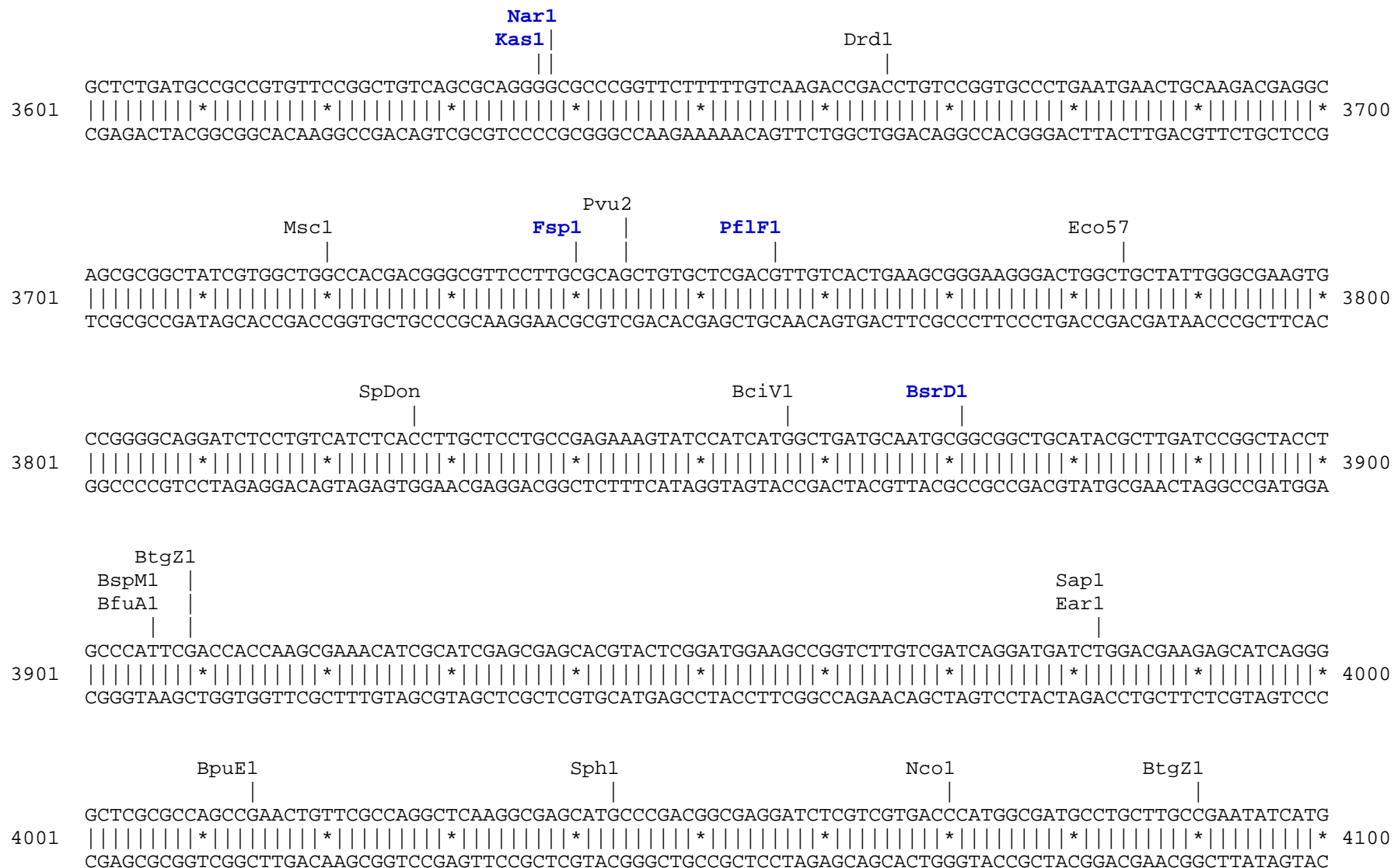
                Nsil
          Sph1 |           Nsil
          BfrB1 |           |
          BstAP |           |
          |     |           |
3201 AAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAAC
   |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3300
   TTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTTCAGGGGTCCGAGGGGTTCGTCCGTCTTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTG

                Ecil
          Ecil   Ecil   Bmr1 |           Ecil   Nco1           polyA
          |     |     | |           |           |           |
3301 CATAGTCCCGCCCTAACTCCGCCCATCCCGCCCTAACTCCGCCCAGTTCCGCCCATTTCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGCAGAG
   |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3400
   GTATCAGGGCGGGGATTGAGGCGGGTAGGGCGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGCGGGGTACCGACTGATTAATAAAAAATAAATACGTCTC

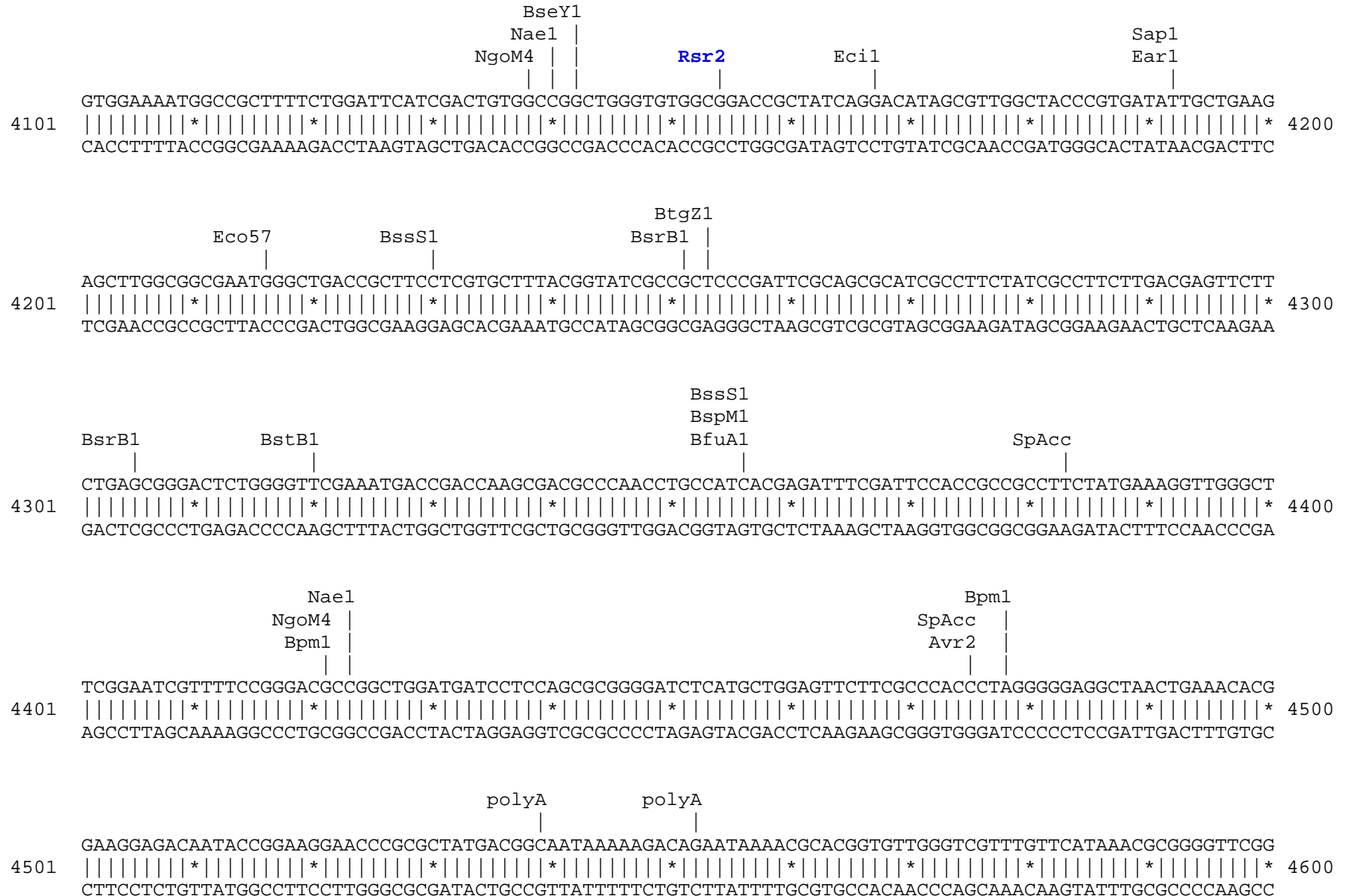
                Avr2
          SfiI |           SpDon |           BseR1 | |           ClaI |           BsaB1
          |     |           |           | |           |           |           |
3401 GCCGAGGCCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGC AAAGATCGATCAAGAGACAGGATGAGGA
   |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3500
   CGGCTCCGGCGGAGCCGGAGACTCGATAAGGTCTTCATCACTCTCCGAAAAACCTCCGGATCCGAAAACGTTTCTAGCTAGTTCTCTGTCTACTCTCT

                BspM1
          BfuA1 |           Eag1 |           Bmr1
          |     |           |           |
3501 TCGTTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCT
   |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 3600
   AGCAAAGCGTACTAACTTGTCTACCTAACGTGCGTCCAAGAGGCCGGCGAACCACCTCTCCGATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGA

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                Bsa1
                |
4601 TCCCAGGGCTGGCACTCTGTTCGATAACCCACCCGAGACCCCATTTGGGGCCAATACGCCCGCGTTTTCTTCTTTTTCCCCACCCACCCCAAGTTCGGGTG
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 4700
    AGGGTCCCGACCGTGAGACAGCTATGGGGTGGCTCTGGGGTAACCCCGTTATGCGGGCGCAAAGAAGGAAAAGGGGTGGGGTGGGGGGTTCAAGCCCAC

                BstAP
                AlwN1      Bsu36                                Dra1
                |          |                                |
4701 AAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGCCTCAGGTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATT
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 4800
    TTCCGGGTCCCGAGCGTCGGTTGCAGCCCCGCCGTCCGGGACGGTATCGGAGTCCAATGAGTATATATGAAATCTAACTAAATTTTGAAGTAAAAATTAA

                Dra1
                |
                BspH1
                |
4801 TAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATC
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 4900
    ATTTTCCTAGATCCACTTCTAGGAAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAG

                BpuE1
                |
4901 AAAGGATCTTCTTGAGATCCTTTTTTTCTGCGGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTTGTTTGCCGGATCAAGAGC
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 5000
    TTTCTAGAAAGAACTCTAGGAAAAAAGACGCGCATTAGACGACGAACGTTTGTTTTTTTGGTGGCGATGGTTCGCCACCAAACAAACGGCCTAGTTCTCG

                Eco57
                |
                SpAcc
                |
5001 TACCAACTCTTTTTCCGAAGGTAAGTGGCTTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTC
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 5100
    ATGGTTGAGAAAAAGGCTTCCATTGACCGAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGATCACATCGGCATCAATCCGGTGGTGAAGTTCTTGAG

                AlwN1
                |
                BpuE1
                |
5101 TGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAG
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 5200
    ACATCGTGGCGGATGTATGGAGCGAGACGATTAGGACAATGGTCACCGACGACGGTCACCGCTATTTCAGCACAGAATGGCCCAACCTGAGTTCTGCTATC

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                                     ApaL1      BseY1
                                     |          |
5201 TTACCGGATAAGGCGCAGCGGTCTGGGCTGAACGGGGGGTTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTG
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 5300
    AATGGCCTATTCCGCGTCGCCAGCCCCGACTTGCCCCCAAGCACGTGTGTCTGGGTCTGAACCTCGCTTGCTGGATGTGGCTTGACTCTATGGATGTCTGCAC

                                     BciV1
                                     |
    SpAcc      Eci1      |          BssS1
    |          |          |          |
5301 AGCTATGAGAAAAGCGCCACGCTTCCCCGAAGGGAGAAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCTGGAACAGGAGAGCGCACGAGGGAGCTTCCAGG
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 5400
    TCGATACTCTTTCGCGGTGCGAAGGGCTTCCCTCTTTCGCTGTCCATAGGCCATTGCGCGTCCCAGCCTTGTCTCTCGCGTGCTCCCTCGAAGGTCC

                                     SpAcc      Drd1      BpuE1      SpAcc
                                     |          |          |          |
5401 GGGAAACGCCTGGTATCTTTATAGTCCTGTCTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGGAAA
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 5500
    CCTTTGCGGACCATAGAAATATCAGGACAGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAAAAACTACGAGCAGTCCCCCGCCTCGGATACCTTT

                                     SpDon
                                     |
    Eci1      |          BspLU
    |          |          |
5501 AACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGTCTGCATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTA
    |||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||*|||||* 5600
    TTGCGGTCGTTGCGCCGGAAAAATGCCAAGGACCGGAAAACGACCGGAAAACGAGTGTACAAGAAAGGACGCAATAGGGGACTAAGACACCTATTGGCAT

                                     NsiI
    BfrB1 |
    |     |
5601 TTACCGCCATGCAT 5614
    |||||*|||||
    AATGGCGGTACGTA
```

Found:

Aat2	<b>Afe1</b>	<b>Afl2</b>	<b>Age1</b>	Ale1	AlwN1	ApaL1	<b>Ase1</b>	Avr2	<b>BamH1</b>	Bbs1	BciV1	BfrB1	BfuA1
Bgl1	<b>Bgl2</b>	Bmr1	Bpm1	BpuE1	Bsa1	BsaB1	<b>BsaXa</b>	<b>BsaXb</b>	BseR1	BseY1	Bsm1	BspH1	<b>BspLU</b>
BspM1	BsrB1	<b>BsrD1</b>	BsrG1	BssS1	BstAP	BstB1	BstX1	Bsu36	BtgZ1	Bts1	<b>_Chi</b>	<b>Cla1</b>	Dra1
Dra3	Drd1	Eag1	Ear1	Ecil	Eco57	<b>EcoN1</b>	<b>EcoR1</b>	<b>Fsp1</b>	<b>Hind3</b>	<b>Hpa1</b>	<b>Kas1</b>	<b>Mfe1</b>	Msc1
Nae1	<b>Nar1</b>	Nco1	<b>Nde1</b>	NgoM4	Nhe1	<b>Not1</b>	Nsi1	<b>PflF1</b>	<b>PflM1</b>	<b>Pml1</b>	polyA	PshA1	Psi1
Pst1	Pvu2	<b>Rsr2</b>	<b>Sac1</b>	<b>SanD1</b>	Sap1	<b>SexA1</b>	<b>Sfil</b>	<b>SnaB1</b>	SpAcc	SpDon	Sph1	Ssp1	<b>Stu1</b>
<b>Xba1</b>	<b>Xcm1</b>	<b>Xho1</b>											

Unique:

<b>Afe1</b>	<b>Afl2</b>	<b>Age1</b>	<b>Ase1</b>	<b>BamH1</b>	<b>Bgl2</b>	<b>BsaXa</b>	<b>BsaXb</b>	<b>BspLU</b>	<b>BsrD1</b>	<b>_Chi</b>	<b>Cla1</b>	<b>EcoN1</b>	<b>EcoR1</b>
<b>Fsp1</b>	<b>Hind3</b>	<b>Hpa1</b>	<b>Kas1</b>	<b>Mfe1</b>	<b>Nar1</b>	<b>Nde1</b>	<b>Not1</b>	<b>PflF1</b>	<b>PflM1</b>	<b>Pml1</b>	<b>Rsr2</b>	<b>Sac1</b>	<b>SanD1</b>
<b>SexA1</b>	<b>Sfil</b>	<b>SnaB1</b>	<b>Stu1</b>	<b>Xba1</b>	<b>Xcm1</b>	<b>Xho1</b>							

Not found:

Aar1	Acc65	Ac11	Ahd1	Apa1	Asc1	AsiS1	Baela	Baelb	BbvC1	Bcgl1a	Bcgl1b	Bcl1	Blp1
BmgB1	Bpu10	Bsg1	BsiW1	BsmB1	BspE1	BssH2	BstE2	BstZ1	EcoK	EcoRV	FCatB	FCatL	FCatR
FCatP	ScFRT	Fse1	FspA1	I_Ceu	Kpn1	loxP	Mlu1	Nru1	Pac1	Pme1	PspOM	Pvu1	R4atB
R4atL	R4atP	R4atR	Sac2	Sall	Sbf1	Sca1	Sgf1	SgrA1	Sma1	Spe1	Srf1	Swal	T3RNA
T7RNA	T7Ter	PISce	Xmn1										

Excluded by site complexity:

Acc1	Acil	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	Faul	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													