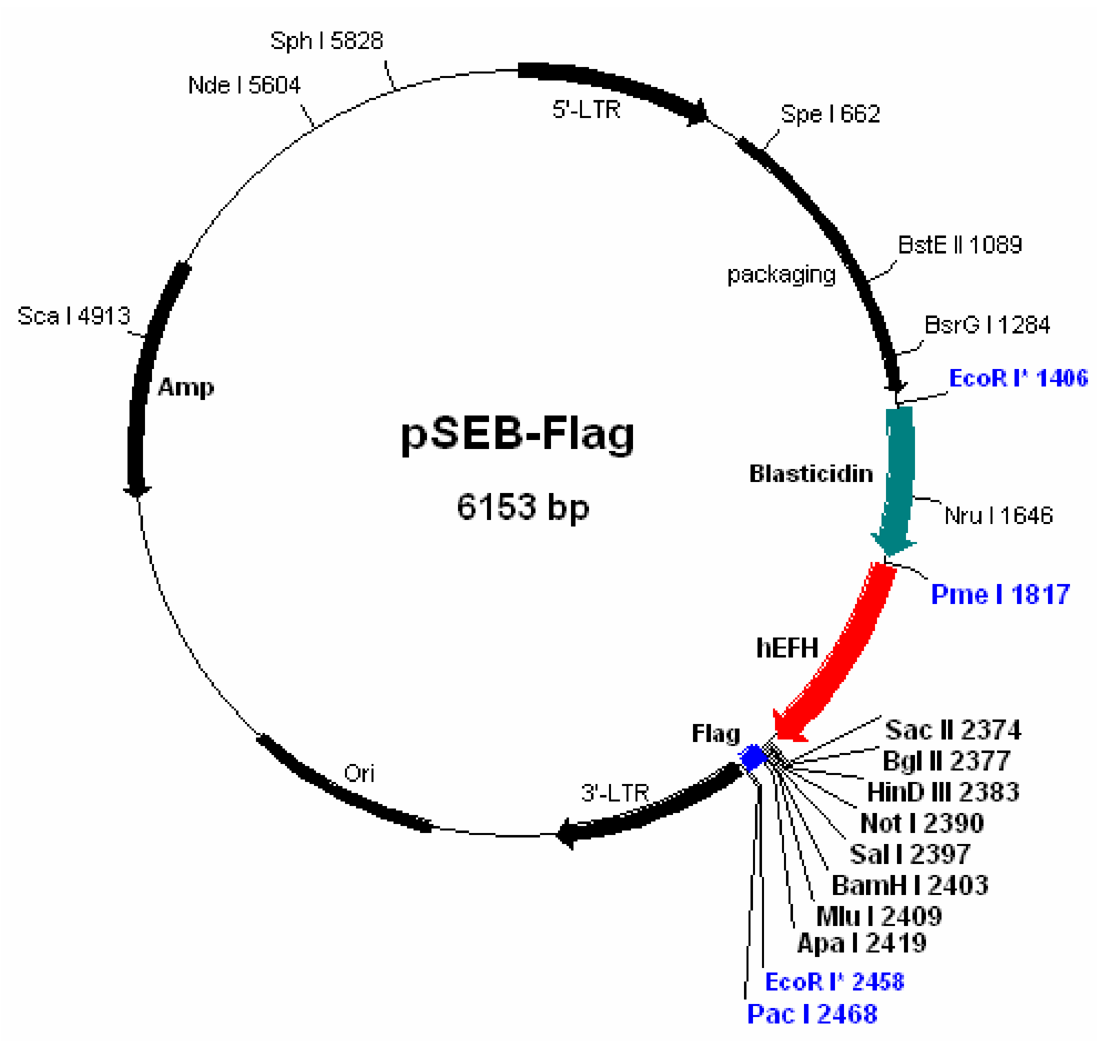
Vector: pSEB-Flag

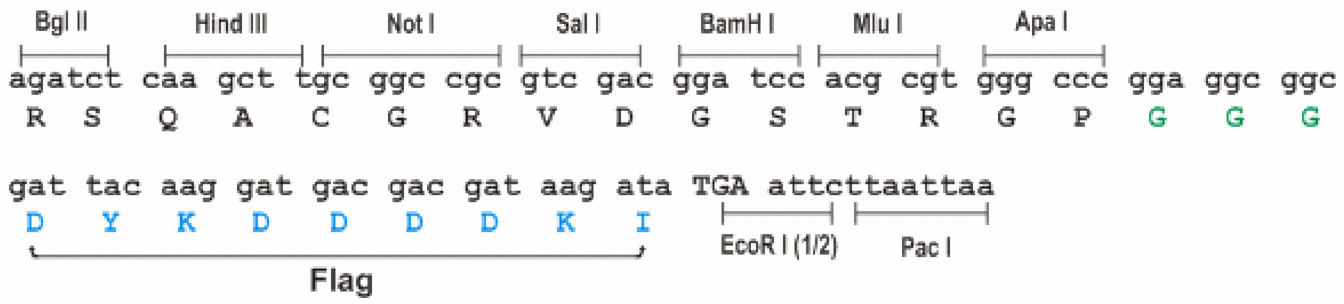
Antibiotic Selection: Amp

Creator(s): Chris Song & Hong Yin, Molecular Oncology Lab of The University of Chicago

Date of Construction: Dec, 2005



Linker Sequence



pSEB-Flag (human EF1a/HLTV hybrid promoter) Full-Length Sequence

TGAAAGACCC CACCTGTAGG TTTGGCAAGC TAGCTTAAGT AACGCCATTT TGCAAGGCATGGAAAATACATAACTGAGAA TAGAGAAGTT AGATCAAGG
TTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGC
CCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCT
GTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGCGCGCCAGTCCTCCGATAGACTGCGTCGCCCGGGTACCCGTATTCCCAAT
AAAGCCTCTTG CTGTTTGCAT CCGAATCGTG GACTCGCTGA TCCTTGGGAG GGTCTCCTCAGATTGATTGACTGCCCACCTCGGGGGTCTTTCATTTGGAG
GTTCCACCGA GATTTGGAGACCCCTGCCCA GGGACCACCGACCCCCCCGC CGGGAGGTAA GCTGGCCAGC GGTCGTTTCGTGTCTGTCTC TGTCTTTGTG
CGTGTTTGTGCCGGCATCTAATGTTTGCGCCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAACTGACGAGTTCTGAACACCCG
GCCG CAACCCTGGG AGACGTCCCAGGGACTTTGGGGGCCGTTTTTGTGGCCCGACCTGAGGAAGGGAGTCGATGTGGAATCCGACCCCGTCAGGATATGTGGTT
CTGGTAGGAGACGAGAACCT AAAACAGTTC CCGCCTCCGT CTGAATTTTTGCTTTCGGTT TGGAACCGAAGCCGCGCGTC TTGTCTGCTG CAGCGCTGCA
GCATCGTTCT GTGTTGTCTC TGTCTGACTGTGTTTCTGTA TTTGTCTGAA AATTAGGGCC AGACTGTTAC CACTCCCTTAAGTTTGACCTTAGGTCACTG
GAAAGATGTCGAGCGGATCG CTCACAACCA GTCGGTAGAT GTCAAGAAGAGACGTTGGGT TACCTTCTGC TCTGCAGAAT GGCCAACCTTTAACGTCGGA
TGGCCGCGAGACGGCACCTT TAACCGAGAC CTCATCACCC AGGTTAAGAT CAAGGTCTTT TCACCTGGCCCGCATGGACA CCCAGACCAGGTCCCCTACA
TCGTGACCTG GGAAGCCTTG GCTTTTGACCCCCCTCCCTG GGTCAAGCCCTTTGTACACC CTAAGCCTCC GCCTCCTCTT CCTCCATCCGCCCCGTCTCT
CCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCG**GAATTC**ACCATGGCCAAGCCTTTGTCTCAAGA
AGAATCCACCCTCATTGAAAGAGCAACGGCTACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAGCGACGGCCGCATCTTC
ACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGTGCAGAACTCGTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGA
TCGGAAATGAGAACAGGGGCATCTTGAGCCCCTGCGGACGGTGCCGACAGGTGCTTCTCGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACA
GCCGACGGCAGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCtaa**gtttaaacatgcag**CGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACAT
CGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCG
CCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTAGCTTCG
AGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACT
GCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGA
CCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTAC **atcgat ccgcgg AGAtctaagctt**
gcgg ccg cgt cga cgg atc cA CGC GT GGGCCC gga ggc ggc gat tac aag gat gac gac gat aag ata TGA attc**ttaattaa**
CGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATG
GAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGG
CTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGT
GCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGCGCGCCAGTC
CTCCGATAGACTGCGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGA
TTGACTACCCGTCAGCGGGGGTCTTTCATGGGTAACAGTTTCTTGAAGTTGGAGAACAACATTCTGAGGGTAGGAGTCGAATATTAAGTAATCCTGACTCAATTAG
CCACTGTTTTGAATCCACATACTCCAATACTCCTGAAATAGTTCATTATGGACAGCGCAGAAGAGCTGGGGAGAATTAATTCGTAATCATGGTCATAGCTGTTTCC
TGTG TGAAATTGTTATCCGCTCAC AATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTG
CGTTGCGCTC ACTGCCCGCT TTCCAGTCGGGAAACCTGTC GTGCCAGCTGCATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTTGCGTATTGGGCG
CTCTTCCGCT TCCTCGCTCA CTGACTCGCTGCGCTCGGTC GTTCGGCTGCGGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTTATCCACAGAA
TCAGGGGATAACGCAGGAAA GAACATGTGAGCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCGCGTTGCTGGC GTTTTTCCATAGGCTCCGCC
CCCCTGACGA GCATCACAAA AATCGACGCTCAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATACCAGGCGTTT CCCCCTGGAAGCTCCCTCGT
GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTCTCCCTTCGGG AAGCGTGGCGCTTTCTCATA GCTCACGCTGTAGGTATCTC
AGTTCGGTGTAGGTCGTTCG CTCCAAGCTGGGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCGCCTTATCCGG TAACTATCGT CTTGAGTCCA
ACCCGGTAAGACACGACTTA TCGCCACTGGCAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCTTGAAGTGGTG
GCCTAACTACGGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGCTGAAGCCAGT TACCTTCGGAAAAAGAGTTG GTAGCTCTTG ATCCGGCAAA
CAAACCACCGCTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTACGCGCAGAAAA AAAGGATCTCAAGAAGATCC TTTGATCTTT TCTACGGGGT
CTGACGCTCA GTGGAACGAA AACTCACGTTAAGGGATTTT GGTCATGAGATTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAAAATGAAGTTT
TAAATCAATCTAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAATGCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATTTCGTTCATCC
ATAGTTGCCTGACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTGCAATGATACC GCGAGACCCACGCTCACCGG
CTCCAGATTT ATCAGCAATA AACCAGCCAGCCGGAAGGGC CGAGCGCAGAAGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTAATTGTTGCCG
GGAAGCTAGA GTAAGTAGTT CGCCAGTTAATAGTTTGCGC AACGTTGTTGCCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGGTATGGCTTCA
TTCAGCTCCGGTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCTCCTTCGGTCC TCCGATCGTTGTCAGAAGTA
AGTTGGCCGC AGTGTTATCA CTCATGGTTATGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGTAAGATGCTTT TCTGTGACTGGTGAGTACTC
AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCCCGGCGTCAAT ACGGGATAAT ACCGCGCCACATAGCAGAACTTTAAAAGTG
CTCATCATTGGAAAACGTTC TTCGGGGCGAAAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGATGTAACCCAC TCGTGCACCC AACTGATCTT
CAGCATCTTTTACTTTCACC AGCGTTTCTGGGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAATGTTGAATACT
CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTCTCATGAGCGG ATACATATTTGAATGTATTT AGAAAAATAAACAAATAGGG
GTTCCGCGCACATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCATTATTATCATG ACATTAACCTATAAAAATAG GCGTATCACG AGGCCCTTTC
GTCTCGCGCG TTTCGGTGAT GACGGTGAAAACCTCTGACA CATGCAGCTC CCGGAGACGG TCACAGCTTG TCTGTAAGCGGATGCCGGGAGCAGACAAGC
CCGTCAGGGCGCGTCAGCGG GTGTTGGCGG GTGTCGGGGC TGGCTTAACTATGCGGCATC AGAGCAGATT GTACTGAGAG TGCACCATATGCGGTGTGAA
ATACCGCACAGATGCGTAAG GAGAAAATAC CGCATCAGGC GCCATTCGCC ATTCAGGCTG CGCAACTGTTGGGAAGGGCG ATCGGTGCGGGCCTCTTCGC
TATTACGCCA GCTGGCGAAA GGGGGATGTGCTGCAAGGCG ATTAAGTTGGGTAACGCCAG GGTTTTCCCA GTCACGACGT TGTAAAACGACGGCGCAAGG
AATGGTGCAT GCAAGGAGAT GGCGCCCAACAGTCCCCCGG CCACGGGGCCTGCCACCATA CCCACGCCGA AACAAGCGCT CATGAGCCCGAAGTGGCGAG
CCCGATCTTCCCCATCGGTG ATGTCGGCGA TATAGGCGCC AGCAACCGCA CCTGTGGCGC CGGTGATGCCGGCCACGATG CGTCCGGCGTAGAGGCGATT
AGTCCAATTT GTTAAAGACA GGATATCAGTGGTCCAGGCT CTAGTTTTGA CTCAACAATA TCACCAGCTGAAGCCTATAG AGTACGAGCCATAGATAAAA
TAAAAGATTT TATTTAGTCT CCAGAAAAAG GGGGGAA

Unique enzymes in pSEB-Flag:

Spe I	**A`CTAG,T**	**662**
BstE II	**G`GTNAC,C**	**1089**
BsrG I	**T`GTAC,A**	**1284**
Nco I	**C`CATG,G**	**1413**
Bbv II	**GAAGAC 7/11**	**1499**
Bbs I	**GAAGAC 8/12**	**1500**
Nru I	**TCG|CGA**	**1646**
BsaB I	**GATNN|NNATC**	**1713**
Pme I	**CTTT|AAAC**	**1817**
Mun I	**C`AATT,G**	**1899**
Age I	**A`CCGG,T**	**1906**
Cla I	**AT`CG,AT**	**2366**
Sac II	**CC,GC`GG**	**2374**
Bgl II	**A`GATC,T**	**2377**
HinD III	**A`AGCT,T**	**2383**
Not I	**GC`GGCC,GC**	**2390**
Sal I	**G`TCGA,C**	**2397**
Acc I	**GT`MK,AC**	**2398**
HinC II	**GTY|RAC**	**2399**
Hind II	**GTY|RAC**	**2399**
BamH I	**G`GATC,C**	**2403**
Mlu I	**A`CGCG,T**	**2409**
Bsp120 I	**G`GGCC,C**	**2415**
Apa I	**G,GGCC`C**	**2419**
Pac I	**TTA,AT`TAA**	**2468**
Sca I	**AGT|ACT**	**4913**
Nde I	**CA`TA,TG**	**5604**
Sph I	**G,CATG`C**	**5828**

Number of enzymes = 28

The following enzymes do not cut in pSEB-Flag:

Avr II   Bcl I   Blp I   BsaA I   BsiC I

```
BsiW I      Bsm I       Bsp1286 I   BspM I      BspM II
Bst1107 I   BstB I      BstX I      Dra III     Eco72 I
Esp I       Fse I       Hpa I       Nsi I       PaeR7 I
PflM I      Pml I       Rsr II      Sfi I       SnaB I
Spl I       Srf I       Stu I       Xca I       Xcm I
```

pSEB-Flag: sites sorted by name:

```
Aat II      (2)   747      5355
Acc I       (1)   2398
Acc65 I     (2)   403      2918
Aci I       (69)  199      205      568      590
                  690      729      862      903
                  1044     1135     1201     1300
                  1319     1357     1534     1616
                  1683     1928     1965     2045
                  2101     2104     2119     2150
                  2157     2184     2371     2373
                  2389     2393     2425     2714
                  2720     3016     3230     3333
                  3389     3399     3423     3466
                  3473     3494     3585     3613
                  3740     3759     3880     3990
                  4125     4134     4496     4587
                  4778     4824     4945     4989
                  5066     5175     5274     5321
                  5495     5534     5544     5570
                  5608     5621     5647     5704
                  5963
Afl II      (3)   35       1008     2550
Afl III     (2)   2409     3540
Age I       (1)   1906
Aha II      (9)   744      1400     2357     4970
                  5352     5656     5839     5953
                  5974
Ahd I       (4)   1022     2963     3009     4433
Alu I       (33)  30       34       338      582
                  670      678      1517     1622
                  2058     2064     2068     2206
                  2347     2385     2545     2549
                  2853     3172     3205     3300
                  3364     3482     3708     3798
                  3844     4101     4622     4722
                  4785     5464     5483     5728
                  6084
Alw I       (16)  455      1053     1359     1729
                  2335     2363     2399     2410
                  4102     4188     4188     4285
                  4286     4750     5065     5071
AlwN I      (3)   159      2674     3956
Apa I       (1)   2419
ApaL I      (3)   3854     5100     5597
Apo I       (3)   874      1406     2458
Asc I       (2)   370      2885
Ase I       (4)   3183     3311     3370     4605
Asp718      (2)   403      2918
Ava I       (7)   333      399      500      1873
                  1975     2848     2914
Ava II      (13)  188      201      256      553
                  692      1221     1574     2703
                  2716     2771     4571     4793
                  6048
BamH I      (1)   2403
Ban I       (17)  246      403      1144     1399
                  1689     1834     1909     2165
                  2356     2761     2918     3284
                  4381     5655     5838     5952
                  5973
Ban II      (9)   340      353      1679     2080
                  2419     2855     2868     5905
                  5919
Bbe I       (6)   1403     2360     5659     5842
                  5956     5977
Bbs I       (1)   1500
Bbv I       (10)  933      941      1526     1631
                  1836     3959     3962     4168
                  4862     5473
Bbv II      (1)   1499
Bcn I       (16)  166      401      402      573
                  726      2221     2420     2681
                  2916     2917     3921     4617
                  4968     5469     5504     5855
Bfa I       (16)  31       221      663      675
                  1396     1524     1914     2065
                  2191     2253     2546     2736
                  4035     4288     4623     6058
Bgl I       (2)   4553     5671
Bgl II      (1)   2377
Bpm I       (3)   2483     4503     6121
Bsa I       (7)   478      532      1151     2209
                  2971     2992     4494
BsaB I      (1)   1713
BsaH I      (9)   744      1400     2357     4970
                  5352     5656     5839     5953
                  5974
BsaJ I      (33)  163      242      252      399
                  463      499      548      549
                  696      735      736      748
                  749      1169     1238     1247
                  1267     1268     1413     1718
                  1976     2240     2371     2678
                  2757     2767     2914     2977
                  3279     3700     5774     5852
                  5858
BsaW I      (5)   1831     1906     3746     3893
                  4724
BseR I      (4)   466      1295     1334     2980
Bsg I       (2)   1602     2024
BsiE I      (10)  594      729      1534     1650
                  2393     3456     3880     4803
                  4952     5699
BsiHKA I    (6)   340      2855     3858     5019
                  5104     5601
BsmA I      (22)  108      477      533      611
                  736      834      951      1075
                  1134     1152     1330     1433
                  2210     2500     2623     2970
                  2991     4495     5269     5422
                  5466     6138
BsmB I      (7)   735      833      1074     1133
                  1331     5423     5465
BsmF I      (11)  174      187      566      731
                  766      1207     1587     1855
                  2689     2702     5834
BsoF I      (42)  729      903      919      922
                  927      930      1135     1515
                  1534     1608     1611     1614
                  1617     1620     1784     1825
                  2045     2104     2119     2150
                  2390     2393     2426     3365
                  3446     3464     3467     3585
                  3740     3883     3948     3951
                  4157     4485     4824     4851
                  4946     5175     5462     5571
                  5675     5748
Bsp120 I    (1)   2415
BspH I      (4)   4260     5268     5373     5897
Bsr I       (18)  376      1032     1060     1548
                  1572     1941     1962     2891
                  3341     3947     3960     4074
                  4480     4598     4641     4908
                  5080     5786
BsrB I      (4)   1044     3232     3473     5274
BsrD I      (2)   4494     4668
BsrG I      (1)   1284
BssH II     (4)   319      370      2834     2885
BssS I      (4)   1589     3713     5097     5404
BstE II     (1)   1089
BstN I      (17)  243      550      737      750
                  1171     1196     1219     1239
                  1269     1719     2758     3280
                  3568     3689     3702     5775
                  6052
BstU I      (26)  319      321      372      905
                  907      1137     1646     1928
                  2097     2142     2373     2395
                  2411     2834     2836     2887
                  3387     3389     3587     4168
                  4498     4991     5323     5423
                  5425     5528
BstY I      (9)   2339     2377     2403     4181
                  4192     4278     4290     5058
                  5075
Bsu36 I     (3)   783      1020     2113
Cac8 I      (37)  28       32       321      372
                  584      588      633      652
                  1201     1510     1624     2066
                  2080     2101     2264     2292
                  2387     2543     2547     2836
                  2887     3333     3362     3471
                  3557     3594     4154     4545
                  5558     5706     5726     5730
                  5826     5867     5915     5957
                  5987
Cfr10 I     (8)   631      1906     2130     2262
                  2353     4513     5976     5985
Cla I       (1)   2366
Csp6 I      (8)   404      660      1285     1956
                  2919     4912     5588     6099
Dde I       (23)  75       169      210      478
                  783      1020     1292     1810
                  2113     2207     2258     2381
                  2590     2684     2725     2994
                  3065     3815     4224     4390
                  4930     5356     5591
Dpn I       (28)  95       461      1048     1180
                  1365     1649     1710     1724
                  2341     2369     2379     2405
                  2610     4108     4183     4194
                  4202     4280     4292     4397
                  4738     4756     4802     5060
                  5077     5113     5698     5922
DpnII       (28)  93       459      1046     1178
                  1363     1647     1708     1722
                  2339     2367     2377     2403
                  2608     4106     4181     4192
                  4200     4278     4290     4395
                  4736     4754     4800     5058
                  5075     5111     5696     5920
Dra I       (5)   1817     2202     4299     4318
                  5010
Drd I       (3)   1502     3648     5517
Dsa I       (4)   696      1413     2371     5858
Eae I       (11)  584      726      1111     1132
                  1416     1531     2390     3379
                  4821     5855     5987
Eag I       (3)   726      1531     2390
Ear I       (6)   1072     1313     3162     3424
                  5228     5716
Eco47 III   (3)   925      1828     5894
Eco57 I     (5)   1511     2079     4087     5101
                  6105
EcoN I      (4)   786      1394     1917     2112
EcoO109 I   (6)   256      1221     1574     2771
                  5409     5863
EcoR I      (2)   1406     2458
EcoR II     (17)  241      548      735      748
                  1169     1194     1217     1237
                  1267     1717     2756     3278
                  3566     3687     3700     5773
                  6050
EcoR V      (3)   141      2656     6041
Ehe I       (6)   1401     2358     5657     5840
                  5954     5975
Fnu4H I     (42)  729      903      919      922
                  927      930      1135     1515
                  1534     1608     1611     1614
                  1617     1620     1784     1825
                  2045     2104     2119     2150
                  2390     2393     2426     3365
                  3446     3464     3467     3585
                  3740     3883     3948     3951
                  4157     4485     4824     4851
                  4946     5175     5462     5571
                  5675     5748
Fok I       (13)  425      1142     1302     1466
                  1701     2109     2451     2939
                  4399     4580     4867     5510
                  5754
Fsp I       (2)   4655     5678
Gdi II      (11)  725      727      1131     1530
                  1532     2389     2391     3380
                  4820     5856     5988
Gsu I       (3)   2484     4504     6122
Hae I       (6)   586      1113     1418     3555
                  3566     4018
```

```
Hae II      (12)  927    1403   1830   2237
                  2360   3418   3788   5659
                  5842   5896   5956   5977
Hae III     (31)  130    175    586    728
                  764    776    989    1113
                  1134   1199   1418   1533
                  2118   2223   2392   2417
                  2645   2690   3381   3555
                  3566   3584   4018   4476
                  4556   4823   5410   5708
                  5857   5865   5989
Hga I       (12)  383    644    897    1493
                  2170   2385   2898   3650
                  4228   4960   5518   5987
HgiA I      (6)   340    2855   3858   5019
                  5104   5601
HgiE II     (2)   4119   5599
Hha I       (43)  321    323    372    374
                  650    907    926    1402
                  1514   1829   1856   1928
                  2099   2236   2331   2359
                  2836   2838   2887   2889
                  3164   3324   3389   3417
                  3450   3720   3787   3887
                  4061   4170   4563   4656
                  4993   5325   5425   5528
                  5658   5679   5812   5841
                  5895   5955   5976
HinC II     (1)   2399
Hind II     (1)   2399
HinD III    (1)   2383
Hinf I      (17)  444    452    793    804
                  1439   1771   2137   2256
                  3075   3097   3118   3375
                  3440   3515   3911   4428
                  6066
HinI I      (9)   744    1400   2357   4970
                  5352   5656   5839   5953
                  5974
HinP I      (43)  319    321    370    372
                  648    905    924    1400
                  1512   1827   1854   1926
                  2097   2234   2329   2357
                  2834   2836   2885   2887
                  3162   3322   3387   3415
                  3448   3718   3785   3885
                  4059   4168   4561   4654
                  4991   5323   5423   5526
                  5656   5677   5810   5839
                  5893   5953   5974
Hpa II      (32)  165    400    571    632
                  725    1403   1832   1907
                  2131   2219   2231   2263
                  2354   2419   2680   2915
                  3258   3747   3894   3920
                  4110   4514   4548   4615
                  4725   4967   5468   5502
                  5854   5977   5986   6001
Hph I       (13)  1159   1185   1403   4277
                  4504   4918   5126   5159
                  5443   5452   5945   5990
                  6071
Kas I       (6)   1399   2356   5655   5838
                  5952   5973
Kpn I       (2)   407    2922
Mae I       (16)  31     221    663    675
                  1396   1524   1914   2065
                  2191   2253   2546   2736
                  4035   4288   4623   6058
Mae II      (10)  744    1083   1124   2021
                  2304   4243   4659   5032
                  5352   5794
Mae III     (20)  39     996    1024   1089
                  1233   2333   2349   2554
                  3032   3896   3959   4075
                  4358   4689   4747   4900
                  5088   5476   5767   5787
Mbo I       (28)  93     459    1046   1178
                  1363   1647   1708   1722
                  2339   2367   2377   2403
                  2608   4106   4181   4192
                  4200   4278   4290   4395
                  4736   4754   4800   5058
                  5075   5111   5696   5920
Mbo II      (14)  1088   1301   1447   1504
                  1532   3178   3412   4201
                  4274   5029   5107   5216
                  5704   5916
Mlu I       (1)   2409
Mme I       (6)   831    1108   2979   3031
                  3754   3938
Mnl I       (52)  218    370    389    434
                  462    486    508    512
                  568    778    874    1170
                  1273   1306   1312   1315
                  1321   1351   1354   1369
                  1376   1392   1456   1797
                  1798   1882   1971   2066
                  2108   2162   2178   2415
                  2733   2885   2904   2949
                  2976   3000   3060   3389
                  3438   3648   3721   3972
                  4372   4453   4599   4805
                  5400   5458   5718   6002
Msc I       (3)   586    1113   1418
Mse I       (26)  36     1009   1121   1151
                  1175   1816   2201   2464
                  2468   2551   3085   3183
                  3311   3370   4246   4298
                  4303   4317   4370   4605
                  4644   5009   5381   5562
                  5759   6029
Msl I       (2)   4685   4844
Msp I       (32)  165    400    571    632
                  725    1403   1832   1907
                  2131   2219   2231   2263
                  2354   2419   2680   2915
                  3258   3747   3894   3920
                  4110   4514   4548   4615
                  4725   4967   5468   5502
                  5854   5977   5986   6001
MspA1 I     (12)  590    1622   2058   2373
                  3016   3364   3882   4127
                  5068   5534   5728   6084
```

```
Mun I       (1)   1899
Nae I       (3)   633    2264   5987
Nar I       (6)   1400   2357   5656   5839
                  5953   5974
Nci I       (16)  165    400    401    572
                  725    2220   2419   2680
                  2915   2916   3920   4616
                  4967   5468   5503   5854
Nco I       (1)   1413
Nde I       (1)   5604
NgoM I      (3)   631    2262   5985
Nhe I       (3)   30     2064   2545
Nla III     (18)  62     1207   1417   1824
                  2577   3031   3198   3544
                  4264   4755   4765   4843
                  4879   5272   5377   5461
                  5828   5901
Nla IV      (39)  190    203    248    405
                  523    554    694    763
                  895    1146   1223   1401
                  1575   1691   1836   1911
                  1963   2167   2246   2358
                  2405   2417   2705   2718
                  2763   2920   3286   3572
                  3611   4383   4477   4518
                  4729   5319   5657   5840
                  5864   5954   5975
Not I       (1)   2390
Nru I       (1)   1646
Nsp7524 I   (4)   1820   3540   5457   5824
NspB II     (12)  590    1622   2058   2373
                  3016   3364   3882   4127
                  5068   5534   5728   6084
NspH I      (4)   1824   3544   5461   5828
Pac I       (1)   2468
Pal I       (31)  130    175    586    728
                  764    776    989    1113
                  1134   1199   1418   1533
                  2118   2223   2392   2417
                  2645   2690   3381   3555
                  3566   3584   4018   4476
                  4556   4823   5410   5708
                  5857   5865   5989
Ple I       (4)   801    2145   3083   3919
Pme I       (1)   1817
PpuM I      (4)   256    1221   1574   2771
Psp1406 I   (3)   2021   4659   5032
PspA I      (2)   399    2914
Pst I       (3)   923    931    1107
Pvu I       (3)   1650   4803   5699
Pvu II      (5)   1622   2058   3364   5728
                  6084
Rsa I       (8)   405    661    1286   1957
                  2920   4913   5589   6100
Sac I       (2)   340    2855
Sac II      (1)   2374
Sal I       (1)   2397
Sap I       (2)   3162   3424
Sau3A I     (28)  93     459    1046   1178
                  1363   1647   1708   1722
                  2339   2367   2377   2403
                  2608   4106   4181   4192
                  4200   4278   4290   4395
                  4736   4754   4800   5058
                  5075   5111   5696   5920
Sau96 I     (29)  128    173    188    201
                  256    553    692    762
                  775    987    1198   1221
                  1574   2221   2415   2416
                  2643   2688   2703   2716
                  2771   4475   4554   4571
                  4793   5409   5706   5863
                  6048
Sca I       (1)   4913
ScrF I      (33)  165    243    400    401
                  550    572    725    737
                  750    1171   1196   1219
                  1239   1269   1719   2220
                  2419   2680   2758   2915
                  2916   3280   3568   3689
                  3702   3920   4616   4967
                  5468   5503   5775   5854
                  6052
Sec I       (33)  163    242    252    399
                  463    499    548    549
                  696    735    736    748
                  749    1169   1238   1247
                  1267   1268   1413   1718
                  1976   2240   2371   2678
                  2757   2767   2914   2977
                  3279   3700   5774   5852
                  5858
SfaN I      (21)  187    446    643    940
                  1487   1544   1676   1722
                  2090   2702   2960   3636
                  4688   4881   5128   5489
                  5581   5619   5657   5973
                  5985
Sfc I       (10)  15     919    927    1103
                  1498   2530   3805   3996
                  4674   6092
Sma I       (2)   401    2916
Spe I       (1)   662
Sph I       (1)   5828
Ssp I       (2)   3083   5237
Sty I       (7)   252    463    1247   1413
                  2240   2767   2977
Taq I       (12)  796    1040   1351   1362
                  1707   2071   2213   2366
                  2398   3078   3640   5084
Tfi I       (7)   444    804    1439   1771
                  3118   3375   3515
Tsp45 I     (7)   1024   1233   2349   4689
                  4900   5476   5787
Tth111 I    (4)   392    813    1219   2907
Tth111 II   (9)   147    424    615    634
                  2280   2662   4129   4138
                  4168
Vsp I       (4)   3183   3311   3370   4605
Xba I       (2)   220    2735
Xho II      (9)   2339   2377   2403   4181
                  4192   4278   4290   5058
                  5075
Xma I       (2)   399    2914
```

Xma III	(3)	726	1531	2390
Xmn I	(3)	3146	3184	5032

Site usage in pSEB-Flag:

Aat II	G,ACGT`C	2	Acc I	GT`MK,AC	1
Acc65 I	G`GTAC,C	2	Aci I	C`CG,C	69
Afl II	C`TTAA,G	3	Afl III	A`CRYG,T	2
Age I	A`CCGG,T	1	Aha II	GR`CG,YC	9
Ahd I	GACNN,N`NNGTC	4	Alu I	AG|CT	33
Alw I	GGATC 8/9	16	AlwN I	CAG,NNN`CTG	3
Apa I	G,GGCC`C	1	ApaL I	G`TGCA,C	3
Apo I	R`AATT,Y	3	Asc I	GG`CGCG,CC	2
Ase I	AT`TA,AT	4	Asp718	G`GTAC,C	2
Ava I	C`YCGR,G	7	Ava II	G`GWC,C	13
Avr II	C`CTAG,G	-	BamH I	G`GATC,C	1
Ban I	G`GYRC,C	17	Ban II	G,RGCY`C	9
Bbe I	G,GCGC`C	6	Bbs I	GAAGAC 8/12	1
Bbv I	GCAGC 13/17	10	Bbv II	GAAGAC 7/11	1
Bcl I	T`GATC,A	-	Bcn I	CC,S`GG	16
Bfa I	C`TA,G	16	Bgl I	GCCN,NNN`NGGC	2
Bgl II	A`GATC,T	1	Blp I	GC`TNA,GC	-
Bpm I	CTGGAG 22/20	3	Bsa I	GGTCTC 7/11	7
BsaA I	YAC|GTR	-	BsaB I	GATNN|NNATC	1
BsaH I	GR`CG,YC	9	BsaJ I	C`CNNG,G	33
BsaW I	W`CCGG,W	5	BseR I	GAGGAG 16/14	4
Bsg I	GTGCAG 22/20	2	BsiC I	TT`CG,AA	-
BsiE I	CG,RY`CG	10	BsiHKA I	G,WGCW`C	6
BsiW I	C`GTAC,G	-	Bsm I	GAATG,C 7	-
BsmA I	GTCTC`/9	22	BsmB I	CGTCTC 7/11	7
BsmF I	GGGAC 15/19	11	BsoF I	GC`N,GC	42
Bsp120 I	G`GGCC,C	1	Bsp1286 I	G,DGCH`C	-
BspH I	T`CATG,A	4	BspM I	ACCTGC 10/14	-
BspM II	T`CCGG,A	-	Bsr I	ACT,GG`	18
BsrB I	GAG|CGG	4	BsrD I	GCAATG, 8	2
BsrG I	T`GTAC,A	1	BssH II	G`CGCG,C	4
BssS I	C`TCGT,G	4	Bst1107 I	GTA|TAC	-
BstB I	TT`CG,AA	-	BstE II	G`GTNAC,C	1
BstN I	CC`W,GG	17	BstU I	CG|CG	26
BstX I	CCAN,NNNN`NTGG	-	BstY I	R`GATC,Y	9
Bsu36 I	CC`TNA,GG	3	Cac8 I	GCN|NGC	37
Cfr10 I	R`CCGG,Y	8	Cla I	AT`CG,AT	1
Csp6 I	G`TA,C	8	Dde I	C`TNA,G	23
Dpn I	GA|TC	28	DpnII	`GATC,	28
Dra I	TTT|AAA	5	Dra III	CAC,NNN`GTG	-
Drd I	GACNN,NN`NNGTC	3	Dsa I	C`CRYG,G	4
Eae I	Y`GGCC,R	11	Eag I	C`GGCC,G	3
Ear I	CTCTTC 7/10	6	Eco47 III	AGC|GCT	3
Eco57 I	CTGAAG 21/19	5	Eco72 I	CAC|GTG	-
EcoN I	CCTNN`N,NNAGG	4	EcoO109 I	RG`GNC,CY	6
EcoR I	G`AATT,C	2	EcoR II	`CCWGG,	17
EcoR V	GAT|ATC	3	Ehe I	GGC|GCC	6
Esp I	GC`TNA,GC	-	Fnu4H I	GC`N,GC	42
Fok I	GGATG 14/18	13	Fse I	GG,CCGG`CC	-
Fsp I	TGC|GCA	2	Gdi II	`YGGC,CG	11
Gsu I	CTGGAG 21/19	3	Hae I	WGG|CCW	6
Hae II	R,GCGC`Y	12	Hae III	GG|CC	31
Hga I	GACGC 9/14	12	HgiA I	G,WGCW`C	6
HgiE II	ACCNNNNNNGGT -1/1	32	Hha I	G,CG`C	43
HinC II	GTY|RAC	1	Hind II	GTY|RAC	1
HinD III	A`AGCT,T	1	Hinf I	G`ANT,C	17
HinI I	GR`CG,YC	9	HinP I	G`CG,C	43
Hpa I	GTT|AAC	-	Hpa II	C`CG,G	32
Hph I	GGTGA 12/11	13	Kas I	G`GCGC,C	6
Kpn I	G,GTAC`C	2	Mae I	C`TA,G	16
Mae II	A`CG,T	10	Mae III	`GTNAC,	20
Mbo I	`GATC,	28	Mbo II	GAAGA 12/11	14
Mlu I	A`CGCG,T	1	Mme I	TCCRAC 25/23	6
Mnl I	CCTC 10/10	52	Msc I	TGG|CCA	3
Mse I	T`TA,A	26	Msl I	CAYNN|NNRTG	2
Msp I	C`CG,G	32	MspA1 I	CMG|CKG	12
Mun I	C`AATT,G	1	Nae I	GCC|GGC	3
Nar I	GG`CG,CC	6	Nci I	CC`S,GG	16
Nco I	C`CATG,G	1	Nde I	CA`TA,TG	1
NgoM I	G`CCGG,C	3	Nhe I	G`CTAG,C	3
Nla III	,CATG`	18	Nla IV	GGN|NCC	39
Not I	GC`GGCC,GC	1	Nru I	TCG|CGA	1
Nsi I	A,TGCA`T	-	Nsp7524 I	R`CATG,Y	4
NspB II	CMG|CKG	12	NspH I	R,CATG`Y	4
Pac I	TTA,AT`TAA	1	PaeR7 I	C`TCGA,G	-
Pal I	GG|CC	31	PflM I	CCAN,NNN`NTGG	-
Ple I	GAGTC 9/10	4	Pme I	CTTT|AAAC	1
Pml I	CAC|GTG	-	PpuM I	RG`GWC,CY	4
Psp1406 I	AA`CG,TT	3	PspA I	C`CCGG,G	2
Pst I	C,TGCA`G	3	Pvu I	CG,AT`CG	3
Pvu II	CAG|CTG	5	Rsa I	GT|AC	8
Rsr II	CG`GWC,CG	-	Sac I	G,AGCT`C	2
Sac II	CC,GC`GG	1	Sal I	G`TCGA,C	1
Sap I	GCTCTTC 8/11	2	Sau3A I	`GATC,	28
Sau96 I	G`GNC,C	29	Sca I	AGT|ACT	1
ScrF I	CC`N,GG	33	Sec I	C`CNNG,G	33
SfaN I	GCATC 9/13	21	Sfc I	C`TRYA,G	10
Sfi I	GGCCN,NNN`NGGCC	-	Sma I	CCC|GGG	2
SnaB I	TAC|GTA	-	Spe I	A`CTAG,T	1
Sph I	G,CATG`C	1	Spl I	C`GTAC,G	-
Srf I	GCCC|GGGC	-	Ssp I	AAT|ATT	2
Stu I	AGG|CCT	-	Sty I	C`CWWG,G	7
Taq I	T`CG,A	12	Tfi I	G`AWT,C	7
Tsp45 I	`GTSAC,	7	Tth111 I	GACN`N,NGTC	4
Tth111 II	CAARCA 16/14	9	Vsp I	AT`TA,AT	4
Xba I	T`CTAG,A	2	Xca I	GTA|TAC	-
Xcm I	CCANNNNN,N`NNNNTGG	-	Xho I	C`TCGA,G	-
Xho II	R`GATC,Y	9	Xma I	C`CCGG,G	2
Xma III	C`GGCC,G	3	Xmn I	GAANN|NNTTC	3