

## pTagBFP-C vector

### Table of restriction sites

| Enzyme name | Sequence                   | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|----------------------------|-------|---|
| AanI        | TTA!TAA                    | 2     | 1514; 1719  |
| AarI        | CACCTGCNNNN!               | 0     |   |
| AarI        | !NNNNNNNNGCAGGTG           | 0     |   |
| AasI        | GACNNNN!NNGTC              | 3     | 1802; 2758; 4547  |
| AatI        | AGG!CCT                    | 2     | 1030; 2552  |
| AatII       | GACGT!C                    | 4     | 125; 178; 261; 447  |
| AbsI        | CC!TCGAGG                  | 0     |   |
| AccI        | GT!MKAC                    | 2     | 1181; 1346  |
| AccII       | CG!CG                      | 16    | 60; 1357; 1619; 1655; 2031; 2051; 2075; 2106; 2797; 3098; 3536; 3620; 3683; 3752; 4025; 4606  |
| AccIII      | T!CCGGA                    | 1     | 1306  |
| Acc16I      | TGC!GCA                    | 1     | 2833  |
| Acc36I      | ACCTGCNNNN!                | 2     | 2999; 3449  |
| Acc36I      | !NNNNNNNNGCAGGT            | 1     | 2618  |
| Acc65I      | G!GTACC                    | 1     | 1351  |
| AccB1I      | G!GYRCC                    | 7     | 465; 655; 715; 1351; 1884; 2730; 2765   |
| AccB7I      | CCANNNN!NTGG               | 0     |   |
| AccBSI      | CCG!CTC                    | 2     | 2155; 3344  |
| AccBSI      | GAG!CGG                    | 2     | 1991; 3398  |
| AceIII      | CAGCTCNNNNNNN!             | 1     | 1687  |
| AceIII      | !NNNNNNNNNNGAGCTG          | 2     | 552; 601  |
| AciI        | C!CGC                      | 35    | 58; 86; 98; 112; 279; 507; 589; 914; 1052; 1154; 1355; 2046; 2049; 2063; 2153; 2401; 2413; 2422; 2434; 2444; 2455; 2501; 2640; 2703; 3205; 3250; 3316; 3342; 3467; 3470; 3618; 3750; 4057; 4201; 4697 |
| AciI        | G!CGG                      | 28    | 370; 403; 528; 595; 749; 1061; 1103; 1357; 1991; 2018; 2106; 2216; 2797; 2861; 2962; 2965; 3245; 3300; 3398; 3536; 3683; 3823; 4066; 4311; 4432; 4451; 4578; 4606                                     |
| AclI        | AA!CGTT                    | 0     |   |
| AclWI       | GGATCNNNN!                 | 9     | 1374; 1383; 2599; 2910; 3156; 3548; 3907; 4005; 4091  |
| AclWI       | !NNNNNGATCC                | 7     | 580; 1136; 1361; 2975; 3519; 3906; 4003   |
| AcoI        | Y!GGCCR                    | 5     | 1259; 2637; 2811; 3202; 3229  |
| AcsI        | R!AATY                     | 4     | 1335; 1545; 1649; 1660  |
| AcuI        | CTGAAGNNNNNNNNNNNNNNN!     | 3     | 1111; 2877; 3309  |
| AcuI        | !NNNNNNNNNNNNNCTTCAG       | 1     | 4107  |
| AcvI        | CAC!GTG                    | 0     |   |
| AcyI        | GR!CGYC                    | 8     | 122; 175; 258; 444; 1178; 2731; 3433; 3512  |
| AdeI        | CACNNN!GTG                 | 2     | 1251; 1847  |
| AfaI        | GT!AC                      | 10    | 219; 244; 299; 332; 383; 540; 646; 1048; 1353; 3037   |
| AfeI        | AGC!GCT                    | 0     |   |
| AfiI        | CCNNNNN!NNGG               | 19    | 49; 712; 825; 1057; 1743; 2069; 2461; 2712; 3256; 3537; 3569; 3701; 3736; 3786; 3818; 4171; 4450; 4616; 4634  |
| AfII        | C!TTAAG                    | 0     |   |
| AfIII       | A!CRYGT                    | 2     | 1617; 4649  |
| AgeI        | A!CCGGT                    | 0     |   |
| AgsI        | TTS!AA                     | 7     | 678; 846; 2142; 2186; 2196; 2609; 4185  |
| AhdI        | GACNNN!NNGTC               | 0     |   |
| AhII        | A!CTAGT                    | 0     |   |
| AjiI        | CAC!GTC                    | 0     |   |
| AjiI        | GAC!GTG                    | 0     |   |
| AjnI        | !CCWGG                     | 17    | 88; 281; 776; 824; 920; 935; 1064; 1173; 2265; 2320; 2337; 3116; 3695; 3798; 4488; 4501; 4622   |
| AjuI        | GAANNNNNNNTTGGNNNNNNNNNNN! | 0     |   |
| AjuI        | !NNNNNNCCAANNNNNNNTTC      | 0     |   |
| AjuI        | CCAANNNNNNNTTCNNNNNNNNNNN! | 0     |   |
| AjuI        | !NNNNNNNGAANNNNNNNTTGG     | 0     |   |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|------------------------------|-------|--|
| AleI        | CACNN!NNGTG                  | 0     |  |
| AlfI        | GCANNNNNNTGCNNNNNNNNNNNN!    | 2     | 2305; 2377   |
| AlfI        | !NNNNNNNNNNGCANNNNNNNTGC     | 2     | 2271; 2343   |
| AloI        | GAACNNNNNNNTCCNNNNNNNNNNNN!  | 2     | 1002; 1813   |
| AloI        | !NNNNNNNNGGANNNNNNNGTTC      | 0     |  |
| AloI        | GGANNNNNNNGTTCNNNNNNNNNNNN!  | 0     |  |
| AloI        | !NNNNNNNGAACNNNNNNNTCC       | 2     | 970; 1781  |
| AluI        | AG!CT                        | 19    | 566; 615; 642; 788; 1095; 1299; 1324; 1330; 1510; 1677; 1934; 2229; 2517; 2837; 3295; 4092; 4349; 4395; 4485                                 |
| AluBI       | AG!CT                        | 19    | 566; 615; 642; 788; 1095; 1299; 1324; 1330; 1510; 1677; 1934; 2229; 2517; 2837; 3295; 4092; 4349; 4395; 4485                                 |
| AlwI        | GGATCNNNN!                   | 9     | 1374; 1383; 2599; 2910; 3156; 3548; 3907; 4005; 4091   |
| AlwI        | !NNNNNGATCC                  | 7     | 580; 1136; 1361; 2975; 3519; 3906; 4003  |
| Alw21I      | GWGCW!C                      | 5     | 685; 1326; 2844; 3034; 4339  |
| Alw26I      | GTCTCN!                      | 1     | 434  |
| Alw26I      | !NNNNNGAGAC                  | 6     | 1033; 1222; 2155; 2573; 3592; 3720   |
| Alw44I      | G!TGCAC                      | 2     | 681; 4335  |
| AlwNI       | CAGNN!CTG                    | 3     | 1268; 3832; 4240   |
| Ama87I      | C!YCGRG                      | 2     | 1319; 1362   |
| Aor13HI     | T!CCGGA                      | 1     | 1306   |
| Aor51HI     | AGC!GCT                      | 0     |  |
| ApaI        | GGGCC!C                      | 1     | 1363   |
| ApaBI       | GCANNNNN!TGC                 | 3     | 2299; 2371; 3833   |
| ApaLI       | G!TGCAC                      | 2     | 681; 4335  |
| ApeKI       | G!CWGC                       | 16    | 800; 944; 1242; 1507; 2026; 2691; 2792; 2834; 2875; 2968; 3355; 3808; 4034; 4240; 4243; 4308   |
| ApoI        | R!AATTY                      | 4     | 1335; 1545; 1649; 1660   |
| ApyPI       | ATCGACNNNNNNNNNNNNNNNNNNNN!  | 1     | 3246   |
| ApyPI       | !NNNNNNNNNNNNNNNNNNGTCGAT    | 3     | 3040; 3693; 4531   |
| AquII       | GCCGNACNNNNNNNNNNNNNNNNNNNN! | 1     | 3130   |
| AquII       | !NNNNNNNNNNNNNNNNNNGTNCGGC   | 0     |  |
| AquIII      | GAGGAGNNNNNNNNNNNNNNNNNNNN!  | 1     | 2559   |
| AquIII      | !NNNNNNNNNNNNNNNNNNCTCCTC    | 0     |  |
| AquIV       | GRGGAAGNNNNNNNNNNNNNNNNNNNN! | 0     |  |
| AquIV       | !NNNNNNNNNNNNNNNNNNCTCCYC    | 2     | 771; 3301  |
| ArsI        | GACNNNNNTTYGNNNNNNNNNNNN!    | 0     |  |
| ArsI        | !NNNNNNCRAANNNNNNNGTC        | 0     |  |
| ArsI        | CRAANNNNNNGTCNNNNNNNNNNNN!   | 0     |  |
| ArsI        | !NNNNNNNNGACNNNNNNNTTYG      | 0     |  |
| AscI        | GG!CGCGCC                    | 0     |  |
| AseI        | AT!TAAT                      | 1     | 7  |
| Asi256I     | G!ATC                        | 24    | 586; 966; 1116; 1142; 1316; 1367; 1376; 1389; 2569; 2573; 2592; 2903; 2981; 3062; 3071; 3149; 3525; 3541; 3900; 3912; 3990; 3998; 4009; 4084 |
| AsiGI       | A!CCGGT                      | 0     |  |
| AsiSI       | GCGAT!CGC                    | 0     |  |
| AspI        | GACN!NNGTC                   | 1     | 2849   |
| Asp700I     | GAANN!NNTTC                  | 0     |  |
| Asp718I     | G!GTACC                      | 1     | 1351   |
| AspA2I      | C!CTAGG                      | 2     | 2553; 3568   |
| AspEI       | GACNNN!NNGTC                 | 0     |  |
| AspLEI      | GCG!C                        | 20    | 1997; 2005; 2031; 2053; 2062; 2075; 2106; 2725; 2733; 2797; 2834; 3100; 3360; 3536; 3622; 4025; 4134; 4308; 4408; 4475                       |
| AspS9I      | G!GNCC                       | 12    | 83; 276; 751; 994; 1086; 1360; 1838; 3247; 3692; 3738; 3796; 3829  |
| AssI        | AGT!ACT                      | 0     |  |
| AsuII       | TT!CGAA                      | 2     | 1333; 3413   |
| AsuC2I      | CC!SGG                       | 6     | 1363; 1364; 2735; 2895; 3508; 4272   |
| AsuHPI      | GGTGANNNNNNNN!               | 4     | 376; 987; 3802; 3918   |
| AsuHPI      | !NNNNNNNTCACC                | 4     | 876; 1026; 1848; 2909  |
| AsuNHI      | G!CTAGC                      | 1     | 591  |
| AvaI        | C!YCGRG                      | 2     | 1319; 1362   |
| AvaII       | G!GWCC                       | 2     | 3247; 3692   |
| AviII       | TGC!GCA                      | 1     | 2833   |
| AvrII       | C!CTAGG                      | 2     | 2553; 3568   |
| AxyI        | CC!TNAGG                     | 3     | 860; 2211; 3843  |

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|-------------|--------------------------|-------|---|
| BaeI        | ACNNNNGTAYCNNNNNNNNNNNN! | 0     |   |
| BaeI        | !NNNNNNNGRTACNNNNNGT     | 0     |   |
| BaeI        | GRTACNNNNGTNNNNNNNNNNNN! | 0     |   |
| BaeI        | !NNNNNNNNNACNNNNNGTAYC   | 0     |   |
| BaeGI       | GKGC!C                   | 8     | 658; 685; 718; 1295; 1363; 2677; 2770; 4339   |
| BalI        | TGG!CCA                  | 2     | 1261; 2813  |
| BamHI       | G!GATCC                  | 1     | 1366  |
| BanI        | G!GYRCC                  | 7     | 465; 655; 715; 1351; 1884; 2730; 2765   |
| BanII       | GRGCY!C                  | 5     | 1326; 1363; 1922; 3096; 3806  |
| BanIII      | AT!CGAT                  | 1     | 2571  |
| BarI        | GAAGNNNNNTACNNNNNNNNNN!  | 0     |   |
| BarI        | !NNNNNNNGTANNNNNNCTTC    | 0     |   |
| BarI        | GTANNNNNCTTCNNNNNNNNNN!  | 0     |   |
| BarI        | !NNNNNNNGAAGNNNNNTAC     | 0     |   |
| BasI        | CCANNNN!NTGG             | 0     |   |
| BauI        | C!ACGAG                  | 3     | 1246; 3449; 4476  |
| BauI        | C!TCGTG                  | 2     | 1096; 3323  |
| BbeI        | GGCGC!C                  | 1     | 2734  |
| Bbr7I       | GAAGACNNNNNN!            | 1     | 907   |
| Bbr7I       | !NNNNNNNNNNNGTCTTC       | 0     |   |
| BbrPI       | CAC!GTG                  | 0     |   |
| BbsI        | GAAGACNN!                | 1     | 902   |
| BbsI        | !NNNNNNGTCTTC            | 0     |   |
| BbuI        | GCATG!C                  | 3     | 2301; 2373; 3136  |
| BbvI        | GCAGCNNNNNN!             | 8     | 812; 1254; 1519; 2804; 2846; 3367; 3820; 4320   |
| BbvI        | !NNNNNNNNNNNGCTGC        | 7     | 931; 2013; 2678; 2862; 2955; 4021; 4227   |
| Bbv12I      | GWGCW!C                  | 5     | 685; 1326; 2844; 3034; 4339   |
| BbvCI       | CC!TCAGC                 | 0     |   |
| BbvCI       | GC!TGAGG                 | 0     |   |
| BccI        | CCATCNNNN!               | 6     | 677; 1118; 1861; 2425; 2951; 3453   |
| BccI        | !NNNNNGATGG              | 3     | 1829; 2609; 3037  |
| BceAI       | ACGGCNNNNNNNNNN!         | 6     | 813; 957; 1008; 1074; 3157; 3644  |
| BceAI       | !NNNNNNNNNNNGCCGT        | 3     | 1872; 2690; 4149  |
| Bcefl       | ACGGCNNNNNNNNNN!         | 6     | 813; 957; 1008; 1074; 3157; 3644  |
| Bcefl       | !NNNNNNNNNNNGCCGT        | 3     | 1873; 2691; 4150  |
| BcgI        | CGANNNNNNTGCNNNNNNNNNN!  | 0     |   |
| BcgI        | !NNNNNNNNNGCANNNNNTCG    | 0     |   |
| BcgI        | GCANNNNNNTCGNNNNNNNNNN!  | 0     |   |
| BcgI        | !NNNNNNNNNCGANNNNNTGC    | 0     |   |
| BciVI       | GTATCCNNNNN!             | 3     | 2160; 2950; 4451  |
| BciVI       | !NNNNNGGATAC             | 0     |   |
| BclI        | T!GATCA                  | 1     | 1388  |
| BcnI        | CC!SGG                   | 6     | 1363; 1364; 2735; 2895; 3508; 4272  |
| BcuI        | A!CTAGT                  | 0     |   |
| BdaI        | TGANNNNNNTCANNNNNNNNNN!  | 2     | 1411; 4584  |
| BdaI        | !NNNNNNNNNTGANNNNNNTCA   | 2     | 1377; 4550  |
| BfaI        | C!TAG                    | 10    | 592; 785; 1280; 1379; 1580; 1998; 2554; 3569; 3903; 4156  |
| BfiI        | ACTGGGNNNN!              | 2     | 1297; 2680  |
| BfiI        | !NNNCCCAGT               | 2     | 289; 2432   |
| BfmI        | C!TRYAG                  | 5     | 1194; 1340; 2066; 4193; 4384  |
| BfoI        | RGCGC!Y                  | 4     | 1998; 2006; 2734; 4409  |
| BfrI        | C!TTAAG                  | 0     |   |
| BfuI        | GTATCCNNNNN!             | 3     | 2160; 2950; 4451  |
| BfuI        | !NNNNNGGATAC             | 0     |   |
| BfuAI       | ACCTGCNNNN!              | 2     | 2999; 3449  |
| BfuAI       | !NNNNNNNGCAGGT           | 1     | 2618  |
| BfuCI       | !GATC                    | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083  |
| BglI        | GCCNNNN!NGGC             | 5     | 90; 212; 283; 1070; 2506  |
| BglII       | A!GATCT                  | 1     | 1315  |
| BisI        | GC!NGC                   | 33    | 750; 801; 945; 1062; 1243; 1508; 2027; 2049; 2063; 2501; 2640; 2692; 2703; 2793; 2798; 2835; 2876; 2963; 2966; 2969; 3205; 3301; 3342; 3356; 3470; 3809; 3824; 4035; 4241; 4244; 4309; 4452; 4607 |

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|-------------|----------------------------|-------|---|
| BlnI        | C!CTAGG                    | 2     | 2553; 3568  |
| BlpI        | GC!TNAGC                   | 0     |   |
| BlsI        | GCN!GC                     | 33    | 751; 802; 946; 1063; 1244; 1509; 2028; 2050; 2064; 2502; 2641; 2693; 2704; 2794; 2799; 2836; 2877; 2964; 2967; 2970; 3206; 3302; 3343; 3357; 3471; 3810; 3825; 4036; 4242; 4245; 4310; 4453; 4608 |
| BmcAI       | AGT!ACT                    | 0     |   |
| Bme18I      | G!GWCC                     | 2     | 3247; 3692  |
| Bme1390I    | CC!NGG                     | 23    | 90; 283; 778; 826; 922; 937; 1066; 1175; 1363; 1364; 2267; 2322; 2339; 2735; 2895; 3118; 3508; 3697; 3800; 4272; 4490; 4503; 4624   |
| BmeRI       | GACNNN!NNGTC               | 0     |   |
| BmeT110I    | C!YCGRG                    | 2     | 1319; 1362  |
| BmgBI       | CAC!GTC                    | 0     |   |
| BmgBI       | GAC!GTG                    | 0     |   |
| BmgT120I    | GG!NCC                     | 12    | 84; 277; 752; 995; 1087; 1361; 1839; 3248; 3693; 3739; 3797; 3830   |
| BmiI        | GGN!NCC                    | 20    | 467; 657; 717; 1108; 1353; 1361; 1368; 1886; 1907; 1919; 2110; 2271; 2343; 2732; 2767; 3615; 3694; 3739; 4582; 4621   |
| BmrI        | ACTGGGNNNNN!               | 2     | 1297; 2680  |
| BmrI        | !NNNNCCCAGT                | 2     | 289; 2432   |
| BmrFI       | CC!NGG                     | 23    | 90; 283; 778; 826; 922; 937; 1066; 1175; 1363; 1364; 2267; 2322; 2339; 2735; 2895; 3118; 3508; 3697; 3800; 4272; 4490; 4503; 4624   |
| BmsI        | GCATCNNNNN!                | 7     | 839; 1547; 2310; 2382; 3030; 3094; 3369   |
| BmsI        | !NNNNNNNNNGATGC            | 7     | 357; 992; 1160; 2689; 2944; 3160; 4552  |
| BmtI        | GCTAG!C                    | 1     | 595   |
| BmuI        | ACTGGGNNNNN!               | 2     | 1297; 2680  |
| BmuI        | !NNNNCCCAGT                | 2     | 289; 2432   |
| BoxI        | GACNN!NNGTC                | 1     | 1234  |
| BpiI        | GAAGACNN!                  | 1     | 902   |
| BpiI        | !NNNNNNGTCTTC              | 0     |   |
| BpII        | GAGNNNNNCTCNNNNNNNNNNNNN!  | 2     | 2520; 3419  |
| BpII        | !NNNNNNNNNGAGNNNNNCTC      | 2     | 2488; 3387  |
| BpmI        | CTGGAGNNNNNNNNNNNNNNNNN!   | 1     | 3571  |
| BpmI        | !NNNNNNNNNNNNNNNCTCCAG     | 2     | 919; 3514   |
| Bpu10I      | CC!TNAGC                   | 0     |   |
| Bpu10I      | GC!TNAGG                   | 0     |   |
| Bpu14I      | TT!CGAA                    | 2     | 1333; 3413  |
| Bpu1102I    | GC!TNAGC                   | 0     |   |
| BpuAI       | GAAGACNN!                  | 1     | 902   |
| BpuAI       | !NNNNNNGTCTTC              | 0     |   |
| BpuEI       | CTTGAGNNNNNNNNNNNNNNNNN!   | 2     | 4025; 4564  |
| BpuEI       | !NNNNNNNNNNNNNNNCTCAAG     | 4     | 1150; 1310; 3107; 4266  |
| BpuMI       | CC!SGG                     | 6     | 1363; 1364; 2735; 2895; 3508; 4272  |
| BpvUI       | CGAT!CG                    | 0     |   |
| BsaI        | GGTCTCN!                   | 0     |   |
| BsaI        | !NNNNNGAGACC               | 2     | 1222; 3720  |
| Bsa29I      | AT!CGAT                    | 1     | 2571  |
| BsaAI       | YAC!GTR                    | 3     | 340; 1847; 3035   |
| BsaBI       | GATNN!NNATC                | 2     | 1393; 2590  |
| BsaHI       | GR!CGYC                    | 8     | 122; 175; 258; 444; 1178; 2731; 3433; 3512  |
| BsaJI       | C!CNNGG                    | 22    | 360; 659; 689; 824; 825; 920; 1355; 1362; 2265; 2337; 2460; 2495; 2504; 2553; 2894; 3163; 3568; 3695; 3696; 3798; 3799; 4489  |
| BsaMI       | GAATGCN!                   | 1     | 1482  |
| BsaMI       | G!CATTC                    | 1     | 1575  |
| BsaWI       | W!CCGGW                    | 6     | 1306; 1372; 2762; 3606; 4296; 4443  |
| BsaXI       | ACNNNNNCTCCNNNNNNNNNNN!    | 1     | 1811  |
| BsaXI       | !NNNNNNGGAGNNNNNNGT        | 0     |   |
| BsaXI       | GGAGNNNNNNGTNNNNNNNNNNN!   | 0     |   |
| BsaXI       | !NNNNNNNNNACNNNNNCTCC      | 1     | 1781  |
| BsbI        | CAACACNNNNNNNNNNNNNNNNNNN! | 0     |   |
| BsbI        | !NNNNNNNNNNNNNNNNNNGTGTTG  | 2     | 1732; 3639  |
| Bsc4I       | CCNNNNN!NNGG               | 19    | 49; 712; 825; 1057; 1743; 2069; 2461; 2712; 3256; 3537; 3569; 3701; 3736; 3786; 3818; 4171; 4450; 4616; 4634  |

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|-------------|-------------------------|-------|---|
| BscAI       | GCATCNNNN!              | 7     | 838; 1546; 2309; 2381; 3029; 3093; 3368   |
| BscAI       | !NNNNNNGATGC            | 7     | 360; 995; 1163; 2692; 2947; 3163; 4555  |
| BseI        | ACTGGN!                 | 5     | 1205; 1292; 2675; 2876; 4122  |
| BseI        | C!CAGT                  | 5     | 295; 1760; 2438; 4234; 4247   |
| Bse8I       | GATNN!NNATC             | 2     | 1393; 2590  |
| Bse2II      | CC!TNAGG                | 3     | 860; 2211; 3843   |
| Bse118I     | R!CCGGY                 | 4     | 1948; 3050; 3231; 3514  |
| BseAI       | T!CCGGA                 | 1     | 1306  |
| BseBI       | CC!WGG                  | 17    | 90; 283; 778; 826; 922; 937; 1066; 1175; 2267; 2322; 2339; 3118; 3697; 3800; 4490; 4503; 4624   |
| BseCI       | AT!CGAT                 | 1     | 2571  |
| BseDI       | C!CNNGG                 | 22    | 360; 659; 689; 824; 825; 920; 1355; 1362; 2265; 2337; 2460; 2495; 2504; 2553; 2894; 3163; 3568; 3695; 3696; 3798; 3799; 4489                                |
| Bse3DI      | GCAATGNN!               | 1     | 2964  |
| Bse3DI      | !CATTGC                 | 0     |   |
| BseGI       | GGATGNN!                | 4     | 2591; 3048; 3073; 3527  |
| BseGI       | !CATCC                  | 5     | 685; 773; 830; 985; 2417  |
| BseJI       | GATNN!NNATC             | 2     | 1393; 2590  |
| BseLI       | CCNNNNN!NNGG            | 19    | 49; 712; 825; 1057; 1743; 2069; 2461; 2712; 3256; 3537; 3569; 3701; 3736; 3786; 3818; 4171; 4450; 4616; 4634  |
| BseMI       | GCAATGNN!               | 1     | 2964  |
| BseMI       | !CATTGC                 | 0     |   |
| BseMII      | CTCAGNNNNNNNNNN!        | 2     | 1326; 3857  |
| BseMII      | !NNNNNNNNCTGAG          | 6     | 851; 2202; 2504; 3385; 3957; 4366   |
| BseNI       | ACTGGN!                 | 5     | 1205; 1292; 2675; 2876; 4122  |
| BseNI       | C!CAGT                  | 5     | 295; 1760; 2438; 4234; 4247   |
| BsePI       | G!CGCGC                 | 0     |   |
| BseRI       | GAGGAGNNNNNNNNNN!       | 1     | 2549  |
| BseRI       | !NNNNNNNNCTCCTC         | 0     |   |
| BseSI       | GKGC!C                  | 8     | 658; 685; 718; 1295; 1363; 2677; 2770; 4339   |
| BseXI       | GCAGCNNNNNNNN!          | 8     | 812; 1254; 1519; 2804; 2846; 3367; 3820; 4320   |
| BseXI       | !NNNNNNNNNNNGCTGC       | 7     | 931; 2013; 2678; 2862; 2955; 4021; 4227   |
| BseX3I      | C!GGCCG                 | 1     | 2637  |
| BseYI       | C!CCAGC                 | 3     | 2274; 2346; 4345  |
| BseYI       | G!CTGGG                 | 2     | 1022; 3235  |
| BsgI        | GTGCAGNNNNNNNNNNNNNNNN! | 0     |   |
| BsgI        | !NNNNNNNNNNNNNNNNCTGCAC | 0     |   |
| Bsh1236I    | CG!CG                   | 16    | 60; 1357; 1619; 1655; 2031; 2051; 2075; 2106; 2797; 3098; 3536; 3620; 3683; 3752; 4025; 4606  |
| Bsh1285I    | CGRY!CG                 | 3     | 599; 2640; 4315   |
| BshFI       | GG!CC                   | 27    | 84; 277; 752; 995; 1030; 1064; 1087; 1219; 1261; 1361; 1697; 1839; 2494; 2500; 2509; 2552; 2639; 2813; 3204; 3231; 3740; 3797; 3830; 4175; 4609; 4627; 4638 |
| BshNI       | G!GYRCC                 | 7     | 465; 655; 715; 1351; 1884; 2730; 2765   |
| BshTI       | A!CCGGT                 | 0     |   |
| BshVI       | AT!CGAT                 | 1     | 2571  |
| BsiEI       | CGRY!CG                 | 3     | 599; 2640; 4315   |
| BsiHKAI     | GWGCW!C                 | 5     | 685; 1326; 2844; 3034; 4339   |
| BsiHKCI     | C!YCGRG                 | 2     | 1319; 1362  |
| BsiSI       | C!CGG                   | 19    | 1307; 1363; 1373; 1949; 2636; 2713; 2735; 2763; 2894; 2984; 3051; 3232; 3507; 3515; 3607; 4081; 4271; 4297; 4444  |
| BsiWI       | C!GTACG                 | 0     |   |
| BsII        | CCNNNNN!NNGG            | 19    | 49; 712; 825; 1057; 1743; 2069; 2461; 2712; 3256; 3537; 3569; 3701; 3736; 3786; 3818; 4171; 4450; 4616; 4634  |
| BsIFI       | GGGACNNNNNNNNNN!        | 6     | 175; 326; 494; 2882; 3414; 3523   |
| BsIFI       | !NNNNNNNNNNNNNGTCCC     | 4     | 2247; 2319; 2383; 3678  |
| BsmI        | GAATGCN!                | 1     | 1482  |
| BsmI        | G!CATTC                 | 1     | 1575  |
| BsmAI       | GTCTCN!                 | 1     | 434   |
| BsmAI       | !NNNNNGAGAC             | 6     | 1033; 1222; 2155; 2573; 3592; 3720  |
| BsmBI       | CGTCTCN!                | 0     |   |
| BsmBI       | !NNNNNGAGACG            | 1     | 1033  |
| BsmFI       | GGGACNNNNNNNNNN!        | 6     | 175; 326; 494; 2882; 3414; 3523   |
| BsmFI       | !NNNNNNNNNNNNNGTCCC     | 4     | 2247; 2319; 2383; 3678  |

| Enzyme name | Sequence               | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|------------------------|-------|---|
| BsnI        | GG!CC                  | 27    | 84; 277; 752; 995; 1030; 1064; 1087; 1219; 1261; 1361; 1697; 1839; 2494; 2500; 2509; 2552; 2639; 2813; 3204; 3231; 3740; 3797; 3830; 4175; 4609; 4627; 4638   |
| Bso31I      | GGTCTCN!               | 0     |   |
| Bso31I      | !NNNNNGAGACC           | 2     | 1222; 3720  |
| BsoBI       | C!YCGRG                | 2     | 1319; 1362  |
| Bsp13I      | T!CCGGA                | 1     | 1306  |
| Bsp19I      | C!CATGG                | 3     | 360; 2460; 3163   |
| Bsp24I      | GACNNNNNTGGNNNNNNNNNN! | 2     | 289; 3534   |
| Bsp24I      | !NNNNNNCCANNNNNGTC     | 2     | 3955; 4133  |
| Bsp24I      | CCANNNNNGTCNNNNNNNNNN! | 2     | 3987; 4165  |
| Bsp24I      | !NNNNNNNGACNNNNNTGG    | 2     | 257; 3502   |
| Bsp68I      | TCG!CGA                | 0     |   |
| Bsp119I     | TT!CGAA                | 2     | 1333; 3413  |
| Bsp120I     | G!GGCCC                | 1     | 1359  |
| Bsp143I     | !GATC                  | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083  |
| Bsp1286I    | GDGCH!C                | 14    | 658; 685; 718; 1295; 1326; 1363; 1922; 2677; 2770; 2844; 3034; 3096; 3806; 4339   |
| Bsp1407I    | T!GTACA                | 1     | 644   |
| Bsp1720I    | GC!TNAGC               | 0     |   |
| BspACI      | C!CGC                  | 35    | 58; 86; 98; 112; 279; 507; 589; 914; 1052; 1154; 1355; 2046; 2049; 2063; 2153; 2401; 2413; 2422; 2434; 2444; 2455; 2501; 2640; 2703; 3205; 3250; 3316; 3342; 3467; 3470; 3618; 3750; 4057; 4201; 4697 |
| BspACI      | G!CGG                  | 28    | 370; 403; 528; 595; 749; 1061; 1103; 1357; 1991; 2018; 2106; 2216; 2797; 2861; 2962; 2965; 3245; 3300; 3398; 3536; 3683; 3823; 4066; 4311; 4432; 4451; 4578; 4606                                     |
| BspCNI      | CTCAGNNNNNNNN!         | 2     | 1325; 3856  |
| BspCNI      | !NNNNNNNCTGAG          | 6     | 852; 2203; 2505; 3386; 3958; 4367   |
| BspDI       | AT!CGAT                | 1     | 2571  |
| BspD6I      | GACTCNNNN!             | 5     | 418; 1318; 1804; 3410; 4287   |
| BspD6I      | !NNNNNGAGTC            | 3     | 874; 1767; 2199   |
| BspEI       | T!CCGGA                | 1     | 1306  |
| BspFNI      | CG!CG                  | 16    | 60; 1357; 1619; 1655; 2031; 2051; 2075; 2106; 2797; 3098; 3536; 3620; 3683; 3752; 4025; 4606  |
| BspHI       | T!CATGA                | 2     | 2157; 3929  |
| BspLI       | GGN!NCC                | 20    | 467; 657; 717; 1108; 1353; 1361; 1368; 1886; 1907; 1919; 2110; 2271; 2343; 2732; 2767; 3615; 3694; 3739; 4582; 4621   |
| BspMI       | ACCTGCNNNN!            | 2     | 2999; 3449  |
| BspMI       | !NNNNNNNGCAGGT         | 1     | 2618  |
| BspOI       | GCTAG!C                | 1     | 595   |
| BspPI       | GGATCNNNN!             | 9     | 1374; 1383; 2599; 2910; 3156; 3548; 3907; 4005; 4091  |
| BspPI       | !NNNNNGATCC            | 7     | 580; 1136; 1361; 2975; 3519; 3906; 4003   |
| BspQI       | GCTCTCN!               | 0     |   |
| BspQI       | !NNNNGAAGAGC           | 2     | 3075; 3285  |
| BspTI       | C!TTAAG                | 0     |   |
| BspT104I    | TT!CGAA                | 2     | 1333; 3413  |
| BspT107I    | G!GYRCC                | 7     | 465; 655; 715; 1351; 1884; 2730; 2765   |
| BspTNI      | GGTCTCN!               | 0     |   |
| BspTNI      | !NNNNNGAGACC           | 2     | 1222; 3720  |
| BsrI        | ACTGGN!                | 5     | 1205; 1292; 2675; 2876; 4122  |
| BsrI        | C!CAGT                 | 5     | 295; 1760; 2438; 4234; 4247   |
| BsrBI       | CCG!CTC                | 2     | 2155; 3344  |
| BsrBI       | GAG!CGG                | 2     | 1991; 3398  |
| BsrDI       | GCAATGNN!              | 1     | 2964  |
| BsrDI       | !CATTGC                | 0     |   |
| BsrFI       | R!CCGGY                | 4     | 1948; 3050; 3231; 3514  |
| BsrGI       | T!GTACA                | 1     | 644   |
| BsrSI       | ACTGGN!                | 5     | 1205; 1292; 2675; 2876; 4122  |
| BsrSI       | C!CAGT                 | 5     | 295; 1760; 2438; 4234; 4247   |
| BssAI       | R!CCGGY                | 4     | 1948; 3050; 3231; 3514  |
| BssECI      | C!CNNGG                | 22    | 360; 659; 689; 824; 825; 920; 1355; 1362; 2265; 2337; 2460; 2495; 2504; 2553; 2894; 3163; 3568; 3695; 3696; 3798; 3799; 4489  |

| Enzyme name | Sequence     | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|--------------|-------|---|
| BssHII      | G!CGCGC      | 0     |   |
| BssKI       | !CCNGG       | 23    | 88; 281; 776; 824; 920; 935; 1064; 1173; 1361; 1362; 2265; 2320; 2337; 2733; 2893; 3116; 3506; 3695; 3798; 4270; 4488; 4501; 4622   |
| BssMI       | !GATC        | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083  |
| BssNI       | GR!CGYC      | 8     | 122; 175; 258; 444; 1178; 2731; 3433; 3512  |
| BssNAI      | GTA!TAC      | 0     |   |
| BssSI       | C!ACGAG      | 3     | 1246; 3449; 4476  |
| BssSI       | C!TCGTG      | 2     | 1096; 3323  |
| BssTII      | C!CWGGG      | 5     | 360; 2460; 2553; 3163; 3568   |
| Bst6I       | CTCTTCN!     | 0     |   |
| Bst6I       | !NNNNGAAGAG  | 3     | 2198; 3075; 3285  |
| Bst98I      | C!TTAAG      | 0     |   |
| Bst1107I    | GTA!TAC      | 0     |   |
| BstACI      | GR!CGYC      | 8     | 122; 175; 258; 444; 1178; 2731; 3433; 3512  |
| BstAFI      | C!TTAAG      | 0     |   |
| BstAPI      | GCANNNN!NTGC | 3     | 2298; 2370; 3832  |
| BstAUI      | T!GTACA      | 1     | 644   |
| BstBI       | TT!CGAA      | 2     | 1333; 3413  |
| Bst2BI      | C!ACGAG      | 3     | 1246; 3449; 4476  |
| Bst2BI      | C!TCGTG      | 2     | 1096; 3323  |
| BstBAI      | YAC!GTR      | 3     | 340; 1847; 3035   |
| Bst4CI      | ACN!GT       | 15    | 76; 198; 269; 543; 581; 660; 1351; 1822; 3111; 3227; 3335; 3658; 4149; 4619; 4690   |
| BstC8I      | GCN!NGC      | 32    | 86; 279; 593; 613; 703; 907; 1359; 1950; 1993; 2007; 2280; 2299; 2352; 2371; 2625; 2811; 3030; 3096; 3102; 3130; 3134; 3175; 3179; 3233; 3516; 3703; 3750; 3806; 3828; 4039; 4599; 4636 |
| BstDEI      | C!TNAG       | 9     | 860; 1157; 1312; 2211; 2513; 3394; 3843; 3966; 4375   |
| BstDSI      | C!CRYGG      | 5     | 360; 659; 1355; 2460; 3163  |
| BstEII      | G!GTNACC     | 0     |   |
| BstENI      | CCTNN!NNNAGG | 0     |   |
| BstF5I      | GGATGNN!     | 4     | 2591; 3048; 3073; 3527  |
| BstF5I      | !CATCC       | 5     | 685; 773; 830; 985; 2417  |
| BstFNI      | CG!CG        | 16    | 60; 1357; 1619; 1655; 2031; 2051; 2075; 2106; 2797; 3098; 3536; 3620; 3683; 3752; 4025; 4606  |
| BstH2I      | RGCGC!Y      | 4     | 1998; 2006; 2734; 4409  |
| BstHHI      | GCG!C        | 20    | 1997; 2005; 2031; 2053; 2062; 2075; 2106; 2725; 2733; 2797; 2834; 3100; 3360; 3536; 3622; 4025; 4134; 4308; 4408; 4475  |
| BstKTI      | GAT!C        | 24    | 588; 968; 1118; 1144; 1318; 1369; 1378; 1391; 2571; 2575; 2594; 2905; 2983; 3064; 3073; 3151; 3527; 3543; 3902; 3914; 3992; 4000; 4011; 4086  |
| BstMAI      | GTCTCN!      | 1     | 434   |
| BstMAI      | !NNNNGAGAC   | 6     | 1033; 1222; 2155; 2573; 3592; 3720  |
| BstMBI      | !GATC        | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083  |
| BstMCI      | CGRY!CG      | 3     | 599; 2640; 4315   |
| BstMWI      | GCNNNNN!NNGC | 27    | 90; 212; 244; 283; 376; 400; 639; 1070; 1958; 1988; 2015; 2057; 2298; 2370; 2421; 2500; 2722; 2806; 2829; 2968; 3091; 3174; 3441; 3628; 3832; 4031; 4603                                |
| BstNI       | CC!WGG       | 17    | 90; 283; 778; 826; 922; 937; 1066; 1175; 2267; 2322; 2339; 3118; 3697; 3800; 4490; 4503; 4624   |
| BstNSI      | RCATG!Y      | 5     | 633; 2301; 2373; 3136; 4653   |
| BstOI       | CC!WGG       | 17    | 90; 283; 778; 826; 922; 937; 1066; 1175; 2267; 2322; 2339; 3118; 3697; 3800; 4490; 4503; 4624   |
| BstPI       | G!GTNACC     | 0     |   |
| BstPAI      | GACNN!NNGTC  | 1     | 1234  |
| BstSCI      | !CCNGG       | 23    | 88; 281; 776; 824; 920; 935; 1064; 1173; 1361; 1362; 2265; 2320; 2337; 2733; 2893; 3116; 3506; 3695; 3798; 4270; 4488; 4501; 4622   |
| BstSFI      | C!TRYAG      | 5     | 1194; 1340; 2066; 4193; 4384  |
| BstSLI      | GKGC!C       | 8     | 658; 685; 718; 1295; 1363; 2677; 2770; 4339   |
| BstSNI      | TAC!GTA      | 1     | 340   |

| Enzyme name | Sequence                       | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|--------------------------------|-------|---|
| BstUI       | CG!CG                          | 16    | 60; 1357; 1619; 1655; 2031; 2051; 2075; 2106; 2797; 3098; 3536; 3620; 3683; 3752; 4025; 4606  |
| Bst2UI      | CC!WGG                         | 17    | 90; 283; 778; 826; 922; 937; 1066; 1175; 2267; 2322; 2339; 3118; 3697; 3800; 4490; 4503; 4624   |
| BstV1I      | GCAGCNNNNNNNN!                 | 8     | 812; 1254; 1519; 2804; 2846; 3367; 3820; 4320   |
| BstV1I      | !NNNNNNNNNNNGCTGC              | 7     | 931; 2013; 2678; 2862; 2955; 4021; 4227   |
| BstV2I      | GAAGACNN!                      | 1     | 902   |
| BstV2I      | !NNNNNNGTCTTC                  | 0     |   |
| BstXI       | CCANNNNN!NTGG                  | 0     |   |
| BstX2I      | R!GATCY                        | 12    | 585; 1141; 1315; 1366; 1375; 2902; 3148; 3540; 3899; 3911; 3997; 4008   |
| BstYI       | R!GATCY                        | 12    | 585; 1141; 1315; 1366; 1375; 2902; 3148; 3540; 3899; 3911; 3997; 4008   |
| BstZI       | C!GGCCG                        | 1     | 2637  |
| BstZ17I     | GTA!TAC                        | 0     |   |
| BsuI        | GTATCCNNNNNN!                  | 3     | 2160; 2950; 4451  |
| BsuI        | !NNNNNGGATAC                   | 0     |   |
| Bsu15I      | AT!CGAT                        | 1     | 2571  |
| Bsu36I      | CC!TNAGG                       | 3     | 860; 2211; 3843   |
| BsuRI       | GG!CC                          | 27    | 84; 277; 752; 995; 1030; 1064; 1087; 1219; 1261; 1361; 1697; 1839; 2494; 2500; 2509; 2552; 2639; 2813; 3204; 3231; 3740; 3797; 3830; 4175; 4609; 4627; 4638                                       |
| BsuTUI      | AT!CGAT                        | 1     | 2571  |
| BtgI        | C!CRYGG                        | 5     | 360; 659; 1355; 2460; 3163  |
| BtgZI       | GCGATGNNNNNNNNNN!              | 2     | 1848; 3183  |
| BtgZI       | !NNNNNNNNNNNNNNNCATCGC         | 3     | 334; 3002; 3346   |
| BthCI       | GCNG!C                         | 33    | 752; 803; 947; 1064; 1245; 1510; 2029; 2051; 2065; 2503; 2642; 2694; 2705; 2795; 2800; 2837; 2878; 2965; 2968; 2971; 3207; 3303; 3344; 3358; 3472; 3811; 3826; 4037; 4243; 4246; 4311; 4454; 4609 |
| BtrI        | CAC!GTC                        | 0     |   |
| BtrI        | GAC!GTG                        | 0     |   |
| BtsI        | GCAGTGNN!                      | 1     | 1262  |
| BtsI        | !CACTGC                        | 1     | 1570  |
| BtsCI       | GGATGNN!                       | 4     | 2591; 3048; 3073; 3527  |
| BtsCI       | !CATCC                         | 5     | 685; 773; 830; 985; 2417  |
| BtuMI       | TCG!CGA                        | 0     |   |
| BveI        | ACCTGCNNNN!                    | 2     | 2999; 3449  |
| BveI        | !NNNNNNNNNGCAGGT               | 1     | 2618  |
| Cac8I       | GCN!NGC                        | 32    | 86; 279; 593; 613; 703; 907; 1359; 1950; 1993; 2007; 2280; 2299; 2352; 2371; 2625; 2811; 3030; 3096; 3102; 3130; 3134; 3175; 3179; 3233; 3516; 3703; 3750; 3806; 3828; 4039; 4599; 4636           |
| CaiI        | CAGNNN!CTG                     | 3     | 1268; 3832; 4240  |
| CciI        | T!CATGA                        | 2     | 2157; 3929  |
| CciNI       | GC!GGCCGC                      | 0     |   |
| CdiI        | CATC!G                         | 5     | 352; 3020; 3025; 3223; 3364   |
| CdiI        | C!GATG                         | 2     | 1834; 3169  |
| CdpI        | GCGGAGNNNNNNNNNNNNNNNNNNNNNN!  | 1     | 4603  |
| CdpI        | !NNNNNNNNNNNNNNNNNNNNCTCCGC    | 3     | 486; 2392; 2434   |
| CelII       | GC!TNAGC                       | 0     |   |
| CfoI        | GCG!C                          | 20    | 1997; 2005; 2031; 2053; 2062; 2075; 2106; 2725; 2733; 2797; 2834; 3100; 3360; 3536; 3622; 4025; 4134; 4308; 4408; 4475  |
| CfrI        | Y!GGCCR                        | 5     | 1259; 2637; 2811; 3202; 3229  |
| Cfr9I       | C!CCGGG                        | 1     | 1362  |
| Cfr10I      | R!CCGGY                        | 4     | 1948; 3050; 3231; 3514  |
| Cfr13I      | G!GNCC                         | 12    | 83; 276; 751; 994; 1086; 1360; 1838; 3247; 3692; 3738; 3796; 3829   |
| Cfr42I      | CCGC!GG                        | 1     | 1358  |
| ChaI        | GATC!                          | 24    | 589; 969; 1119; 1145; 1319; 1370; 1379; 1392; 2572; 2576; 2595; 2906; 2984; 3065; 3074; 3152; 3528; 3544; 3903; 3915; 3993; 4001; 4012; 4087  |
| CjeI        | CCANNNNNNGTNNNNNNNNNNNNNNNN!   | 4     | 2548; 3988; 4088; 4166  |
| CjeI        | !NNNNNNNNNACNNNNNNNTGG         | 7     | 64; 257; 2215; 2310; 3502; 4052; 4607   |
| CjeI        | ACNNNNNNNTGGNNNNNNNNNNNNNNNN!  | 7     | 98; 291; 2249; 2344; 3536; 4086; 4641   |
| CjeI        | !NNNNNNNNCCANNNNNNGT           | 4     | 2514; 3954; 4054; 4132  |
| CjePI       | CCANNNNNNNNTCNNNNNNNNNNNNNNNN! | 2     | 3988; 4166  |



| Enzyme name | Sequence                   | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|----------------------------|-------|--|
| CjePI       | !NNNNNNNNNGANNNNNNNTGG     | 5     | 257; 722; 1067; 3502; 4101   |
| CjePI       | GANNNNNNNTGGNNNNNNNNNNNN!  | 5     | 290; 755; 1100; 3535; 4134   |
| CjePI       | !NNNNNNNCCANNNNNNNTC       | 2     | 3955; 4133   |
| Clal        | AT!CGAT                    | 1     | 2571   |
| CpoI        | CG!GWCCG                   | 1     | 3247   |
| CseI        | GACGCNNNNN!                | 4     | 526; 1050; 3441; 3520  |
| CseI        | !NNNNNNNNNNNGCGTC          | 4     | 1167; 2064; 3959; 4537   |
| CsiI        | A!CCWGGT                   | 1     | 2320   |
| CspI        | CG!GWCCG                   | 1     | 3247   |
| Csp6I       | G!TAC                      | 10    | 218; 243; 298; 331; 382; 539; 645; 1047; 1352; 3036  |
| Csp45I      | TT!CGAA                    | 2     | 1333; 3413   |
| CspAI       | A!CCGGT                    | 0     |  |
| CspCI       | CAANNNNNGTGGNNNNNNNNNNNN!  | 1     | 411  |
| CspCI       | !NNNNNNNNNNCCACNNNNNTTG    | 0     |  |
| CspCI       | CCACNNNNNTTGNNNNNNNNNNNN!  | 0     |  |
| CspCI       | !NNNNNNNNNNNCAANNNNNGTGG   | 1     | 376  |
| CstMI       | AAGGAGNNNNNNNNNNNNNNNNNN!  | 4     | 647; 1238; 2011; 3620  |
| CstMI       | !NNNNNNNNNNNNNNNNNNCTCCTT  | 0     |  |
| CviAII      | C!ATG                      | 22    | 301; 361; 606; 630; 636; 648; 726; 872; 1083; 2158; 2298; 2370; 2461; 2602; 2947; 3133; 3164; 3190; 3546; 3930; 4650; 4701   |
| CviJI       | RG!CY                      | 93    | 42; 84; 93; 277; 566; 615; 642; 705; 752; 781; 788; 866; 932; 944; 995; 1022; 1030; 1064; 1087; 1095; 1109; 1219; 1261; 1299; 1324; 1330; 1361; 1400; 1510; 1677; 1697; 1839; 1920; 1934; 1948; 2229; 2270; 2342; 2465; 2494; 2500; 2509; 2517; 2540; 2552; 2558; 2639; 2656; 2664; 2691; 2716; 2800; 2809; 2813; 2837; 2875; 2951; 2968; 2987; 3050; 3094; 3104; 3121; 3204; 3231; 3235; 3272; 3295; 3311; 3491; 3518; 3579; 3701; 3740; 3797; 3804; 3811; 3830; 3841; 4092; 4121; 4164; 4175; 4240; 4319; 4344; 4349; 4395; 4485; 4583; 4609; 4627; 4638 |
| CviKI-1     | RG!CY                      | 93    | 42; 84; 93; 277; 566; 615; 642; 705; 752; 781; 788; 866; 932; 944; 995; 1022; 1030; 1064; 1087; 1095; 1109; 1219; 1261; 1299; 1324; 1330; 1361; 1400; 1510; 1677; 1697; 1839; 1920; 1934; 1948; 2229; 2270; 2342; 2465; 2494; 2500; 2509; 2517; 2540; 2552; 2558; 2639; 2656; 2664; 2691; 2716; 2800; 2809; 2813; 2837; 2875; 2951; 2968; 2987; 3050; 3094; 3104; 3121; 3204; 3231; 3235; 3272; 3295; 3311; 3491; 3518; 3579; 3701; 3740; 3797; 3804; 3811; 3830; 3841; 4092; 4121; 4164; 4175; 4240; 4319; 4344; 4349; 4395; 4485; 4583; 4609; 4627; 4638 |
| CviQI       | G!TAC                      | 10    | 218; 243; 298; 331; 382; 539; 645; 1047; 1352; 3036  |
| DdeI        | C!TNAG                     | 9     | 860; 1157; 1312; 2211; 2513; 3394; 3843; 3966; 4375  |
| DinI        | GGC!GCC                    | 1     | 2732   |
| DpnI        | GA!TC                      | 24    | 587; 967; 1117; 1143; 1317; 1368; 1377; 1390; 2570; 2574; 2593; 2904; 2982; 3063; 3072; 3150; 3526; 3542; 3901; 3913; 3991; 3999; 4010; 4085   |
| DpnII       | !GATC                      | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083   |
| DraI        | TTT!AAA                    | 3     | 1433; 3875; 3894   |
| DraII       | RG!GNCCY                   | 1     | 3829   |
| DraIII      | CACNNN!GTG                 | 2     | 1251; 1847   |
| DraRI       | CAAGNACNNNNNNNNNNNNNNNNNN! | 2     | 266; 4211  |
| DraRI       | !NNNNNNNNNNNNNNNNNGTCTTG   | 0     |  |
| DrdI        | GACNNN!NNGTC               | 3     | 1802; 2758; 4547   |
| DrdIV       | TACGACNNNNNNNNNNNNNNNNNN!  | 0     |  |
| DrdIV       | !NNNNNNNNNNNNNNNNNGTCGTA   | 1     | 476  |
| DriI        | GACNNN!NNGTC               | 0     |  |
| DseDI       | GACNNN!NNGTC               | 3     | 1802; 2758; 4547   |
| EaeI        | Y!GGCCR                    | 5     | 1259; 2637; 2811; 3202; 3229   |
| EagI        | C!GGCCG                    | 1     | 2637   |
| Eam1104I    | CTCTTCN!                   | 0     |  |
| Eam1104I    | !NNNNGAAGAG                | 3     | 2198; 3075; 3285   |
| Eam1105I    | GACNNN!NNGTC               | 0     |  |
| EarI        | CTCTTCN!                   | 0     |  |
| EarI        | !NNNNGAAGAG                | 3     | 2198; 3075; 3285   |
| EciI        | GGCGANNNNNNNNNNNN!         | 4     | 2231; 3260; 4447; 4593   |
| EciI        | !NNNNNNNNNTCCGCC           | 4     | 496; 2402; 2423; 2444  |

| Enzyme name | Sequence              | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|-----------------------|-------|---|
| Ecl136II    | GAG!CTC               | 1     | 1324  |
| EclXI       | C!GGCCG               | 1     | 2637  |
| Eco24I      | GRGCY!C               | 5     | 1326; 1363; 1922; 3096; 3806  |
| Eco31I      | GGTCTCN!              | 0     |   |
| Eco31I      | !NNNNNGAGACC          | 2     | 1222; 3720  |
| Eco32I      | GAT!ATC               | 0     |   |
| Eco47I      | G!GWCC                | 2     | 3247; 3692  |
| Eco47III    | AGC!GCT               | 0     |   |
| Eco52I      | C!GGCCG               | 1     | 2637  |
| Eco57I      | CTGAAGNNNNNNNNNNNNNN! | 3     | 1111; 2877; 3309  |
| Eco57I      | !NNNNNNNNNNNNNCTTCAG  | 1     | 4107  |
| Eco72I      | CAC!GTG               | 0     |   |
| Eco81I      | CC!TNAGG              | 3     | 860; 2211; 3843   |
| Eco88I      | C!YCGRG               | 2     | 1319; 1362  |
| Eco91I      | G!GTNACC              | 0     |   |
| Eco105I     | TAC!GTA               | 1     | 340   |
| Eco130I     | C!CWWGG               | 5     | 360; 2460; 2553; 3163; 3568   |
| Eco147I     | AGG!CCT               | 2     | 1030; 2552  |
| EcoHI       | !CCSGG                | 6     | 1361; 1362; 2733; 2893; 3506; 4270  |
| EcoICRI     | GAG!CTC               | 1     | 1324  |
| Eco57MI     | CTGRAGNNNNNNNNNNNNNN! | 4     | 1111; 2877; 3309; 3571  |
| Eco57MI     | !NNNNNNNNNNNNNCTYCAG  | 3     | 919; 3514; 4107   |
| EcoNI       | CCTNN!NNNAGG          | 0     |   |
| Eco065I     | G!GTNACC              | 0     |   |
| Eco0109I    | RG!GNCCY              | 1     | 3829  |
| EcoRI       | G!AATTC               | 1     | 1335  |
| EcoRII      | !CCWGG                | 17    | 88; 281; 776; 824; 920; 935; 1064; 1173; 2265; 2320; 2337; 3116; 3695; 3798; 4488; 4501; 4622   |
| EcoRV       | GAT!ATC               | 0     |   |
| EcoT14I     | C!CWWGG               | 5     | 360; 2460; 2553; 3163; 3568   |
| EcoT22I     | ATGCA!T               | 3     | 2303; 2375; 4706  |
| EcoT38I     | GRGCY!C               | 5     | 1326; 1363; 1922; 3096; 3806  |
| Eco53kI     | GAG!CTC               | 1     | 1324  |
| EgeI        | GGC!GCC               | 1     | 2732  |
| EheI        | GGC!GCC               | 1     | 2732  |
| ErhI        | C!CWWGG               | 5     | 360; 2460; 2553; 3163; 3568   |
| EsaBC3I     | TC!GA                 | 17    | 744; 771; 1239; 1321; 1334; 1347; 1881; 2572; 2845; 3001; 3025; 3061; 3223; 3414; 3459; 3714; 4552  |
| Esp3I       | CGTCTCN!              | 0     |   |
| Esp3I       | !NNNNNGAGACG          | 1     | 1033  |
| FaeI        | CATG!                 | 22    | 304; 364; 609; 633; 639; 651; 729; 875; 1086; 2161; 2301; 2373; 2464; 2605; 2950; 3136; 3167; 3193; 3549; 3933; 4653; 4704  |
| Fail        | YA!TR                 | 62    | 39; 46; 50; 67; 138; 146; 234; 236; 291; 302; 310; 362; 555; 557; 607; 631; 637; 649; 727; 873; 891; 1084; 1137; 1139; 1187; 1393; 1403; 1469; 1514; 1719; 2147; 2159; 2290; 2299; 2362; 2371; 2395; 2462; 2486; 2603; 2667; 2948; 2973; 3134; 3165; 3191; 3263; 3479; 3547; 3625; 3677; 3838; 3855; 3857; 3859; 3931; 4209; 4398; 4514; 4587; 4651; 4702 |
| FalI        | AAGNNNNNCTNNNNNNNNNN! | 2     | 870; 3071   |
| FalI        | !NNNNNNNNAAGNNNNNCTT  | 2     | 838; 3039   |
| FaqI        | GGGACNNNNNNNNNN!      | 6     | 175; 326; 494; 2882; 3414; 3523   |
| FaqI        | !NNNNNNNNNNNNNGTCCC   | 4     | 2247; 2319; 2383; 3678  |
| FatI        | !CATG                 | 22    | 300; 360; 605; 629; 635; 647; 725; 871; 1082; 2157; 2297; 2369; 2460; 2601; 2946; 3132; 3163; 3189; 3545; 3929; 4649; 4700  |
| FauI        | CCCGCNNNN!            | 10    | 93; 119; 286; 1059; 1161; 2053; 2408; 2429; 3625; 3757  |
| FauI        | !NNNNNGCGGG           | 7     | 1096; 1350; 1984; 2854; 3391; 3529; 3676  |
| FauNDI      | CA!TATG               | 1     | 234   |
| FbaI        | T!GATCA               | 1     | 1388  |
| FbII        | GT!MKAC               | 2     | 1181; 1346  |
| FmuI        | GGNC!C                | 12    | 86; 279; 754; 997; 1089; 1363; 1841; 3250; 3695; 3741; 3799; 3832   |
| Fnu4HI      | GC!NGC                | 33    | 750; 801; 945; 1062; 1243; 1508; 2027; 2049; 2063; 2501; 2640; 2692; 2703; 2793; 2798; 2835; 2876; 2963; 2966; 2969; 3205; 3301; 3342; 3356; 3470; 3809; 3824; 4035; 4241; 4244; 4309; 4452; 4607   |

| Enzyme name | Sequence                   | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|----------------------------|-------|--|
| FokI        | GGATGNNNNNNNNNN!           | 4     | 2598; 3055; 3080; 3534   |
| FokI        | !NNNNNNNNNNNNNNCATCC       | 5     | 672; 760; 817; 972; 2404   |
| FriOI       | GRGCY!C                    | 5     | 1326; 1363; 1922; 3096; 3806   |
| FseI        | GGCCGG!CC                  | 0     |  |
| FspI        | TGC!GCA                    | 1     | 2833   |
| FspAI       | RTGC!GCAY                  | 0     |  |
| FspBI       | C!TAG                      | 10    | 592; 785; 1280; 1379; 1580; 1998; 2554; 3569; 3903; 4156   |
| FspEI       | CCNNNNNNNNNNNN!            | 156   | 56; 71; 98; 114; 128; 129; 156; 182; 220; 252; 291; 307; 333; 373; 450; 451; 482; 501; 520; 593; 615; 672; 702; 719; 733; 766; 780; 805; 822; 837; 867; 900; 927; 942; 961; 1002; 1044; 1063; 1078; 1101; 1123; 1146; 1166; 1187; 1233; 1275; 1291; 1320; 1368; 1383; 1414; 1453; 1467; 1608; 1703; 1727; 1750; 1773; 1791; 1813; 1834; 1853; 1872; 1901; 1922; 1936; 1962; 2050; 2076; 2125; 2166; 2184; 2223; 2239; 2277; 2334; 2349; 2406; 2426; 2440; 2457; 2471; 2508; 2523; 2566; 2649; 2716; 2747; 2767; 2782; 2827; 2841; 2907; 2921; 2940; 2956; 2997; 3017; 3064; 3114; 3130; 3150; 3175; 3195; 3218; 3245; 3263; 3289; 3329; 3355; 3379; 3435; 3449; 3477; 3520; 3541; 3576; 3620; 3708; 3732; 3754; 3774; 3790; 3811; 3825; 3844; 3928; 3948; 3976; 3991; 4025; 4067; 4094; 4109; 4154; 4178; 4192; 4214; 4240; 4260; 4284; 4310; 4358; 4377; 4396; 4422; 4457; 4502; 4532; 4546; 4597; 4611; 4636; 4652; 4673; 4702 |
| FspEI       | !NNNNNNNNNNNNNNNGG         | 128   | 8; 35; 59; 144; 166; 197; 252; 295; 347; 375; 400; 435; 463; 509; 529; 552; 580; 607; 634; 676; 697; 721; 763; 782; 811; 847; 884; 907; 926; 956; 977; 1004; 1043; 1069; 1088; 1160; 1186; 1234; 1273; 1292; 1334; 1358; 1402; 1502; 1571; 1679; 1728; 1750; 1778; 1814; 1859; 1888; 1924; 1943; 1971; 1991; 2054; 2077; 2185; 2217; 2235; 2264; 2307; 2336; 2447; 2476; 2519; 2540; 2568; 2601; 2621; 2646; 2673; 2698; 2720; 2748; 2774; 2795; 2846; 2867; 2885; 2933; 2969; 3025; 3050; 3074; 3103; 3126; 3150; 3176; 3198; 3217; 3242; 3282; 3318; 3383; 3468; 3492; 3521; 3555; 3576; 3596; 3641; 3668; 3687; 3720; 3772; 3803; 3830; 3882; 3980; 4051; 4096; 4157; 4222; 4256; 4282; 4300; 4336; 4406; 4429; 4451; 4475; 4508; 4556; 4591; 4609; 4666  |
| Fsp4HI      | GC!NGC                     | 33    | 750; 801; 945; 1062; 1243; 1508; 2027; 2049; 2063; 2501; 2640; 2692; 2703; 2793; 2798; 2835; 2876; 2963; 2966; 2969; 3205; 3301; 3342; 3356; 3470; 3809; 3824; 4035; 4241; 4244; 4309; 4452; 4607  |
| GdiII       | C!GGCCR                    | 1     | 2637   |
| GdiII       | Y!GGCCG                    | 3     | 2637; 3202; 3229   |
| GlaI        | GC!GC                      | 20    | 1996; 2004; 2030; 2052; 2061; 2074; 2105; 2724; 2732; 2796; 2833; 3099; 3359; 3535; 3621; 4024; 4133; 4307; 4407; 4474   |
| GluI        | GC!NGC                     | 33    | 750; 801; 945; 1062; 1243; 1508; 2027; 2049; 2063; 2501; 2640; 2692; 2703; 2793; 2798; 2835; 2876; 2963; 2966; 2969; 3205; 3301; 3342; 3356; 3470; 3809; 3824; 4035; 4241; 4244; 4309; 4452; 4607  |
| GsaI        | CCCAG!C                    | 3     | 2278; 2350; 4349   |
| GsaI        | GCTGG!G                    | 2     | 1026; 3239   |
| GsuI        | CTGGAGNNNNNNNNNNNNNNNN!    | 1     | 3571   |
| GsuI        | !NNNNNNNNNNNNNNCTCCAG      | 2     | 919; 3514  |
| HaeI        | WGG!CCW                    | 8     | 1030; 1219; 1261; 2552; 2813; 4175; 4627; 4638   |
| HaeII       | RGCGC!Y                    | 4     | 1998; 2006; 2734; 4409   |
| HaeIII      | GG!CC                      | 27    | 84; 277; 752; 995; 1030; 1064; 1087; 1219; 1261; 1361; 1697; 1839; 2494; 2500; 2509; 2552; 2639; 2813; 3204; 3231; 3740; 3797; 3830; 4175; 4609; 4627; 4638  |
| HaeIV       | GAYNNNNNRTCNNNNNNNNNNNNNN! | 2     | 1406; 2927   |
| HaeIV       | !NNNNNNNNNGAYNNNNNRTC      | 2     | 1372; 2893   |
| HaeIV       | GAYNNNNNRTCNNNNNNNNNNNNNN! | 2     | 1405; 2926   |
| HaeIV       | !NNNNNNNGAYNNNNNRTC        | 2     | 1374; 2895   |
| HapII       | C!CGG                      | 19    | 1307; 1363; 1373; 1949; 2636; 2713; 2735; 2763; 2894; 2984; 3051; 3232; 3507; 3515; 3607; 4081; 4271; 4297; 4444   |
| HgaI        | GACGCNNNNN!                | 4     | 526; 1050; 3441; 3520  |
| HgaI        | !NNNNNNNNNNNGCGTC          | 4     | 1167; 2064; 3959; 4537   |
| HhaI        | GCG!C                      | 20    | 1997; 2005; 2031; 2053; 2062; 2075; 2106; 2725; 2733; 2797; 2834; 3100; 3360; 3536; 3622; 4025; 4134; 4308; 4408; 4475   |
| HinI        | GR!CGYC                    | 8     | 122; 175; 258; 444; 1178; 2731; 3433; 3512   |
| HinIII      | CATG!                      | 22    | 304; 364; 609; 633; 639; 651; 729; 875; 1086; 2161; 2301; 2373; 2464; 2605; 2950; 3136; 3167; 3193; 3549; 3933; 4653; 4704   |

| Enzyme name | Sequence                 | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------------|-------|--|
| Hin4I       | GAYNNNNNVTCNNNNNNNNNNNN! | 2     | 1405; 2926   |
| Hin4I       | !NNNNNNNGABNNNNNRTC      | 3     | 1373; 2894; 2924   |
| Hin4I       | GABNNNNNRTCNNNNNNNNNNNN! | 3     | 1405; 2926; 2956   |
| Hin4I       | !NNNNNNNGAYNNNNNVTC      | 2     | 1373; 2894   |
| Hin6I       | G!CGC                    | 20    | 1995; 2003; 2029; 2051; 2060; 2073; 2104; 2723; 2731; 2795; 2832; 3098; 3358; 3534; 3620; 4023; 4132; 4306; 4406; 4473   |
| HinP1I      | G!CGC                    | 20    | 1995; 2003; 2029; 2051; 2060; 2073; 2104; 2723; 2731; 2795; 2832; 3098; 3358; 3534; 3620; 4023; 4132; 4306; 4406; 4473   |
| HincII      | GTY!RAC                  | 2     | 1347; 1494   |
| HindII      | GTY!RAC                  | 2     | 1347; 1494   |
| HindIII     | A!AGCTT                  | 2     | 1297; 1328   |
| HinfI       | G!ANTC                   | 15    | 410; 731; 881; 1208; 1310; 1774; 1796; 2206; 3216; 3350; 3402; 3460; 3497; 4279; 4675  |
| HpaI        | GTT!AAC                  | 1     | 1494   |
| HpaII       | C!CGG                    | 19    | 1307; 1363; 1373; 1949; 2636; 2713; 2735; 2763; 2894; 2984; 3051; 3232; 3507; 3515; 3607; 4081; 4271; 4297; 4444   |
| HphI        | GGTGANNNNNNNN!           | 4     | 376; 987; 3802; 3918   |
| HphI        | !NNNNNNNTCACC            | 4     | 876; 1026; 1848; 2909  |
| Hpy8I       | GTN!NAC                  | 14    | 201; 539; 577; 663; 683; 978; 1182; 1191; 1347; 1494; 1778; 1795; 1850; 4337   |
| Hpy99I      | CGWCG!                   | 9     | 1240; 1351; 2821; 2849; 3143; 3158; 3435; 3820; 4553   |
| Hpy166II    | GTN!NAC                  | 14    | 201; 539; 577; 663; 683; 978; 1182; 1191; 1347; 1494; 1778; 1795; 1850; 4337   |
| Hpy188I     | TCN!GA                   | 15    | 585; 690; 970; 1115; 1315; 1905; 2514; 2698; 3042; 3395; 3496; 3975; 4109; 4462; 4540  |
| Hpy188III   | TC!NNGA                  | 25    | 963; 1128; 1167; 1307; 1319; 1379; 2158; 2210; 2523; 2576; 2749; 3065; 3074; 3157; 3213; 3257; 3347; 3380; 3449; 3930; 4004; 4087; 4185; 4283; 4417  |
| HpyAV       | CCTTCNNNNNN!             | 7     | 771; 819; 864; 1041; 3376; 3483; 4162  |
| HpyAV       | !NNNNNGAAGG              | 9     | 691; 1063; 1964; 2859; 3588; 3604; 3787; 4104; 4414  |
| HpyCH4III   | ACN!GT                   | 15    | 76; 198; 269; 543; 581; 660; 1351; 1822; 3111; 3227; 3335; 3658; 4149; 4619; 4690  |
| HpyCH4IV    | A!CGT                    | 16    | 122; 134; 175; 258; 339; 444; 959; 1235; 1791; 1803; 1846; 1956; 2847; 3034; 3815; 3948  |
| HpyCH4V     | TG!CA                    | 20    | 633; 683; 1005; 1342; 1480; 1507; 1575; 2292; 2301; 2364; 2373; 2488; 2564; 2623; 2782; 2957; 2971; 4041; 4337; 4704   |
| HpyF3I      | C!TNAG                   | 9     | 860; 1157; 1312; 2211; 2513; 3394; 3843; 3966; 4375  |
| HpyF10VI    | GCNNNNN!NNGC             | 27    | 90; 212; 244; 283; 376; 400; 639; 1070; 1958; 1988; 2015; 2057; 2298; 2370; 2421; 2500; 2722; 2806; 2829; 2968; 3091; 3174; 3441; 3628; 3832; 4031; 4603   |
| Hsp92I      | GR!CGYC                  | 8     | 122; 175; 258; 444; 1178; 2731; 3433; 3512   |
| Hsp92II     | CATG!                    | 22    | 304; 364; 609; 633; 639; 651; 729; 875; 1086; 2161; 2301; 2373; 2464; 2605; 2950; 3136; 3167; 3193; 3549; 3933; 4653; 4704   |
| HspAI       | G!CGC                    | 20    | 1995; 2003; 2029; 2051; 2060; 2073; 2104; 2723; 2731; 2795; 2832; 3098; 3358; 3534; 3620; 4023; 4132; 4306; 4406; 4473   |
| KasI        | G!GCGCC                  | 1     | 2730   |
| KfII        | GG!GWCCC                 | 0     |  |
| KpnI        | GGTAC!C                  | 1     | 1355   |
| Kpn2I       | T!CCGGA                  | 1     | 1306   |
| KspI        | CCGC!GG                  | 1     | 1358   |
| Ksp22I      | T!GATCA                  | 1     | 1388   |
| KspAI       | GTT!AAC                  | 1     | 1494   |
| Kzo9I       | !GATC                    | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083   |
| LguI        | GCTCTCN!                 | 0     |  |
| LguI        | !NNNNGAAGAGC             | 2     | 3075; 3285   |
| LpnI        | RG!GCGY                  | 4     | 1996; 2004; 2732; 4407   |
| LpnPI       | CCDGNNNNNNNNNN!          | 59    | 102; 295; 733; 790; 838; 872; 934; 949; 1010; 1078; 1102; 1187; 1275; 1320; 1376; 1468; 1773; 1962; 2185; 2223; 2239; 2279; 2334; 2351; 2451; 2536; 2649; 2726; 2748; 2771; 2907; 2921; 2940; 2997; 3064; 3114; 3130; 3187; 3245; 3454; 3520; 3544; 3620; 3709; 3812; 3845; 4076; 4094; 4240; 4260; 4284; 4310; 4359; 4457; 4502; 4532; 4611; 4636; 4673 |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|------------------------------|-------|---|
| LpnPI       | !NNNNNNNNNNNNNNCHGG          | 54    | 75; 268; 552; 763; 811; 907; 1008; 1051; 1160; 1186; 1273; 1292; 1348; 1814; 1934; 1991; 2054; 2252; 2264; 2324; 2336; 2568; 2613; 2656; 2698; 2720; 2748; 2795; 2857; 2879; 2969; 3036; 3059; 3103; 3198; 3217; 3242; 3391; 3492; 3535; 3592; 3682; 3785; 3812; 3830; 4066; 4103; 4256; 4282; 4422; 4440; 4475; 4556; 4609 |
| Lsp1109I    | GCAGCNNNNNNNN!               | 8     | 812; 1254; 1519; 2804; 2846; 3367; 3820; 4320   |
| Lsp1109I    | !NNNNNNNNNNNNNGCTGC          | 7     | 931; 2013; 2678; 2862; 2955; 4021; 4227   |
| LweI        | GCATCNNNNN!                  | 7     | 839; 1547; 2310; 2382; 3030; 3094; 3369   |
| LweI        | !NNNNNNNNNGATGC              | 7     | 357; 992; 1160; 2689; 2944; 3160; 4552  |
| MabI        | A!CCWGGT                     | 1     | 2320  |
| MaeI        | C!TAG                        | 10    | 592; 785; 1280; 1379; 1580; 1998; 2554; 3569; 3903; 4156  |
| MaeII       | A!CGT                        | 16    | 122; 134; 175; 258; 339; 444; 959; 1235; 1791; 1803; 1846; 1956; 2847; 3034; 3815; 3948   |
| MaeIII      | !GTNAC                       | 13    | 61; 148; 497; 882; 1519; 2020; 2032; 2851; 3157; 3847; 4113; 4229; 4292   |
| MalI        | GA!TC                        | 24    | 587; 967; 1117; 1143; 1317; 1368; 1377; 1390; 2570; 2574; 2593; 2904; 2982; 3063; 3072; 3150; 3526; 3542; 3901; 3913; 3991; 3999; 4010; 4085  |
| MaqI        | CRTTGACNNNNNNNNNNNNNNNNNNNN! | 4     | 144; 197; 466; 540  |
| MaqI        | !NNNNNNNNNNNNNNNNNNNGTCAAYG  | 3     | 157; 240; 426   |
| MauBI       | CG!CGCGCG                    | 0     |   |
| MbiI        | CCG!CTC                      | 2     | 2155; 3344  |
| MbiI        | GAG!CGG                      | 2     | 1991; 3398  |
| MboI        | !GATC                        | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083  |
| MboII       | GAAGANNNNNNNN!               | 7     | 907; 1020; 1987; 2215; 3092; 3302; 3921   |
| MboII       | !NNNNNNNTCTTC                | 5     | 834; 3382; 3549; 3749; 3992   |
| McaTI       | GCGC!GC                      | 0     |   |
| MfeI        | C!AATTG                      | 1     | 1481  |
| MfiI        | R!GATCY                      | 12    | 585; 1141; 1315; 1366; 1375; 2902; 3148; 3540; 3899; 3911; 3997; 4008   |
| MhII        | GDGCH!C                      | 14    | 658; 685; 718; 1295; 1326; 1363; 1922; 2677; 2770; 2844; 3034; 3096; 3806; 4339   |
| MlsI        | TGG!CCA                      | 2     | 1261; 2813  |
| MluI        | A!CGCGT                      | 1     | 1617  |
| MluNI       | TGG!CCA                      | 2     | 1261; 2813  |
| MlyI        | GAGTCNNNNN!                  | 3     | 890; 1783; 2215   |
| MlyI        | !NNNNNGACTC                  | 5     | 404; 1304; 1790; 3396; 4273   |
| Mly113I     | GG!CGCC                      | 1     | 2731  |
| MmeI        | TCCRACNNNNNNNNNNNNNNNNNNNN!  | 2     | 1013; 1824  |
| MmeI        | !NNNNNNNNNNNNNNNNNNNGTYGGA   | 2     | 4256; 4440  |
| MnII        | CCTCNNNNNNN!                 | 13    | 764; 802; 943; 958; 1174; 1285; 1450; 2514; 3332; 3538; 3852; 4222; 4546  |
| MnII        | !NNNNNGAGG                   | 22    | 541; 645; 684; 705; 738; 855; 964; 1020; 1209; 1242; 1410; 1875; 2206; 2484; 2527; 2542; 2582; 2646; 2782; 3139; 3569; 4472   |
| Mph1103I    | ATGCA!T                      | 3     | 2303; 2375; 4706  |
| MreI        | CG!CCGGCG                    | 0     |   |
| MroI        | T!CCGGA                      | 1     | 1306  |
| MroNI       | G!CCGGC                      | 3     | 1948; 3231; 3514  |
| MroXI       | GAANN!NNTTC                  | 0     |   |
| MscI        | TGG!CCA                      | 2     | 1261; 2813  |
| MseI        | T!TAA                        | 17    | 7; 620; 1301; 1432; 1493; 1614; 1635; 1646; 1658; 1669; 1686; 1784; 2055; 3874; 3888; 3893; 3945  |
| MslI        | CAYNN!NNRTG                  | 3     | 365; 634; 3168  |
| MspI        | C!CGG                        | 19    | 1307; 1363; 1373; 1949; 2636; 2713; 2735; 2763; 2894; 2984; 3051; 3232; 3507; 3515; 3607; 4081; 4271; 4297; 4444  |
| Msp20I      | TGG!CCA                      | 2     | 1261; 2813  |
| MspA1I      | CMG!CKG                      | 6     | 1054; 1357; 2229; 2837; 4066; 4311  |
| MspCI       | C!TTAAG                      | 0     |   |

| Enzyme name | Sequence                   | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|----------------------------|-------|---|
| MspJI       | CNNRNNNNNNNNNN!            | 239   | 36; 50; 71; 97; 113; 127; 147; 167; 219; 236; 251; 290; 306; 319; 332; 352; 373; 407; 424; 449; 481; 500; 523; 548; 574; 596; 618; 642; 660; 690; 708; 723; 738; 756; 789; 816; 832; 847; 871; 884; 900; 918; 932; 948; 961; 975; 996; 1009; 1030; 1049; 1063; 1077; 1095; 1108; 1126; 1140; 1157; 1177; 1209; 1224; 1239; 1258; 1274; 1291; 1306; 1319; 1333; 1346; 1368; 1385; 1399; 1414; 1438; 1455; 1468; 1509; 1523; 1536; 1554; 1592; 1607; 1625; 1666; 1690; 1710; 1728; 1749; 1772; 1804; 1819; 1841; 1859; 1872; 1893; 1910; 1923; 1936; 1952; 1965; 1995; 2010; 2035; 2053; 2066; 2081; 2105; 2119; 2143; 2168; 2184; 2222; 2238; 2277; 2291; 2305; 2333; 2349; 2363; 2377; 2406; 2419; 2440; 2449; 2471; 2501; 2517; 2535; 2565; 2588; 2612; 2636; 2655; 2683; 2709; 2725; 2738; 2761; 2775; 2795; 2808; 2822; 2841; 2855; 2868; 2884; 2899; 2912; 2928; 2943; 2959; 2984; 3003; 3019; 3032; 3045; 3063; 3077; 3091; 3107; 3129; 3145; 3164; 3186; 3202; 3225; 3244; 3258; 3274; 3288; 3308; 3324; 3337; 3359; 3392; 3406; 3426; 3439; 3453; 3507; 3520; 3543; 3556; 3575; 3592; 3619; 3655; 3688; 3707; 3732; 3753; 3780; 3793; 3810; 3824; 3839; 3855; 3915; 3940; 3956; 3978; 3991; 4005; 4036; 4050; 4075; 4093; 4108; 4121; 4140; 4153; 4168; 4197; 4216; 4234; 4259; 4274; 4293; 4309; 4328; 4346; 4362; 4376; 4395; 4422; 4445; 4467; 4486; 4501; 4514; 4531; 4551; 4578; 4591; 4610; 4635; 4658; 4672; 4685; 4702 |
| MspJI       | !NNNNNNNNNNNNYNG           | 198   | 8; 35; 59; 76; 97; 123; 145; 168; 197; 221; 269; 281; 310; 330; 347; 377; 400; 437; 465; 492; 511; 531; 553; 574; 592; 616; 634; 664; 688; 706; 730; 749; 771; 790; 811; 832; 858; 878; 907; 949; 983; 1004; 1024; 1051; 1051; 1069; 1086; 1114; 1132; 1153; 1172; 1198; 1220; 1248; 1266; 1292; 1313; 1342; 1359; 1383; 1412; 1441; 1483; 1566; 1604; 1628; 1651; 1680; 1724; 1746; 1778; 1814; 1831; 1852; 1889; 1909; 1926; 1943; 1978; 1996; 2014; 2031; 2055; 2077; 2104; 2132; 2158; 2196; 2217; 2234; 2252; 2275; 2296; 2324; 2347; 2368; 2386; 2407; 2424; 2447; 2471; 2491; 2509; 2531; 2562; 2584; 2610; 2629; 2646; 2674; 2693; 2712; 2730; 2748; 2769; 2791; 2815; 2848; 2868; 2886; 2907; 2933; 2960; 2977; 2994; 3011; 3037; 3059; 3081; 3099; 3119; 3138; 3160; 3198; 3218; 3242; 3262; 3282; 3309; 3333; 3366; 3385; 3413; 3435; 3464; 3492; 3517; 3536; 3555; 3576; 3593; 3610; 3641; 3668; 3688; 3711; 3731; 3768; 3785; 3804; 3830; 3850; 3889; 3916; 3935; 3953; 3990; 4008; 4049; 4067; 4093; 4110; 4142; 4171; 4199; 4220; 4243; 4262; 4283; 4301; 4318; 4336; 4362; 4383; 4403; 4423; 4441; 4462; 4488; 4505; 4524; 4543; 4572; 4591; 4609; 4628; 4646; 4664; 4687   |
| MspR9I      | CC!NGG                     | 23    | 90; 283; 778; 826; 922; 937; 1066; 1175; 1363; 1364; 2267; 2322; 2339; 2735; 2895; 3118; 3508; 3697; 3800; 4272; 4490; 4503; 4624   |
| MssI        | GTTT!AAAC                  | 0     |   |
| MunI        | C!AATTG                    | 1     | 1481  |
| MvaI        | CC!WGG                     | 17    | 90; 283; 778; 826; 922; 937; 1066; 1175; 2267; 2322; 2339; 3118; 3697; 3800; 4490; 4503; 4624   |
| MvaI269I    | GAATGCN!                   | 1     | 1482  |
| MvaI269I    | G!CATTC                    | 1     | 1575  |
| MvnI        | CG!CG                      | 16    | 60; 1357; 1619; 1655; 2031; 2051; 2075; 2106; 2797; 3098; 3536; 3620; 3683; 3752; 4025; 4606  |
| MvrI        | CGAT!CG                    | 0     |   |
| MwoI        | GCNNNNN!NNGC               | 27    | 90; 212; 244; 283; 376; 400; 639; 1070; 1958; 1988; 2015; 2057; 2298; 2370; 2421; 2500; 2722; 2806; 2829; 2968; 3091; 3174; 3441; 3628; 3832; 4031; 4603  |
| NaeI        | GCC!GGC                    | 3     | 1950; 3233; 3516  |
| NarI        | GG!CGCC                    | 1     | 2731  |
| NciI        | CC!SGG                     | 6     | 1363; 1364; 2735; 2895; 3508; 4272  |
| NcoI        | C!CATGG                    | 3     | 360; 2460; 3163   |
| NdeI        | CA!TATG                    | 1     | 234   |
| NdeII       | !GATC                      | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083  |
| NgoAVIII    | GACNNNNNTGANNNNNNNNNNNN!   | 1     | 280   |
| NgoAVIII    | !NNNNNNNNNNNTCANNNNNGTC    | 1     | 722   |
| NgoAVIII    | TCANNNNNGTCNNNNNNNNNNNNNN! | 1     | 758   |
| NgoAVIII    | !NNNNNNNNNNNGACNNNNNTGA    | 1     | 244   |
| NgoMIV      | G!CCGGC                    | 3     | 1948; 3231; 3514  |
| NheI        | G!CTAGC                    | 1     | 591   |

| Enzyme name | Sequence                  | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|---------------------------|-------|---|
| NlaIII      | CATG!                     | 22    | 304; 364; 609; 633; 639; 651; 729; 875; 1086; 2161; 2301; 2373; 2464; 2605; 2950; 3136; 3167; 3193; 3549; 3933; 4653; 4704                                  |
| NlaIV       | GGN!NCC                   | 20    | 467; 657; 717; 1108; 1353; 1361; 1368; 1886; 1907; 1919; 2110; 2271; 2343; 2732; 2767; 3615; 3694; 3739; 4582; 4621   |
| NlaCI       | CATCACNNNNNNNNNNNNNNNNNN! | 4     | 694; 1563; 1878; 3470   |
| NlaCI       | !NNNNNNNNNNNNNNNNNGTGATG  | 3     | 347; 982; 4542  |
| Nli3877I    | CYCGR!G                   | 2     | 1323; 1366  |
| NmeAIII     | GCCGAGNNNNNNNNNNNNNNNNNN! | 2     | 2520; 2956  |
| NmeAIII     | !NNNNNNNNNNNNNNNNNNCTCGGC | 2     | 998; 2485   |
| NmeDI       | RCCGGYNNNNNNNN!           | 4     | 1960; 3062; 3243; 3526  |
| NmeDI       | !NNNNNNNNNNNNRCCGGY       | 4     | 1935; 3037; 3218; 3501  |
| NmuCI       | !GTSAC                    | 4     | 882; 2020; 2851; 3157   |
| NotI        | GC!GGCCGC                 | 0     |   |
| NruI        | TCG!CGA                   | 0     |   |
| NsbI        | TGC!GCA                   | 1     | 2833  |
| NsiI        | ATGCA!T                   | 3     | 2303; 2375; 4706  |
| NspI        | RCATG!Y                   | 5     | 633; 2301; 2373; 3136; 4653   |
| NspV        | TT!CGAA                   | 2     | 1333; 3413  |
| OliI        | CACNN!NNGTG               | 0     |   |
| PabI        | GTA!C                     | 10    | 220; 245; 300; 333; 384; 541; 647; 1049; 1354; 3038   |
| PacI        | TTAAT!TAA                 | 0     |   |
| PaeI        | GCATG!C                   | 3     | 2301; 2373; 3136  |
| PaeR7I      | C!TCGAG                   | 1     | 1319  |
| PagI        | T!CATGA                   | 2     | 2157; 3929  |
| PalAI       | GG!CGCGCC                 | 0     |   |
| PasI        | CC!CWGGG                  | 3     | 825; 3696; 3799   |
| PauI        | G!CGCGC                   | 0     |   |
| PceI        | AGG!CCT                   | 2     | 1030; 2552  |
| PciI        | A!CATGT                   | 1     | 4649  |
| PciSI       | GCTCTTCN!                 | 0     |   |
| PciSI       | !NNNNGAAGAGC              | 2     | 3075; 3285  |
| PcsI        | WCGNNNN!NNNCGW            | 7     | 1232; 3025; 3102; 3345; 3419; 3812; 4330  |
| PctI        | GAATGCN!                  | 1     | 1482  |
| PctI        | G!CATTC                   | 1     | 1575  |
| PdiI        | GCC!GGC                   | 3     | 1950; 3233; 3516  |
| PdmI        | GAANN!NNTTC               | 0     |   |
| PfeI        | G!AWTC                    | 7     | 731; 1208; 3216; 3350; 3460; 3497; 4675   |
| Pfl23II     | C!GTACG                   | 0     |   |
| PfIFI       | GACN!NNGTC                | 1     | 2849  |
| PfIMI       | CCANNNN!NTGG              | 0     |   |
| PfoI        | T!CCNGGA                  | 2     | 935; 3506   |
| PhoI        | GG!CC                     | 27    | 84; 277; 752; 995; 1030; 1064; 1087; 1219; 1261; 1361; 1697; 1839; 2494; 2500; 2509; 2552; 2639; 2813; 3204; 3231; 3740; 3797; 3830; 4175; 4609; 4627; 4638 |
| PinAI       | A!CCGGT                   | 0     |   |
| PlaDI       | CATCAGNNNNNNNNNNNNNNNNNN! | 1     | 3112  |
| PlaDI       | !NNNNNNNNNNNNNNNNNNCTGATG | 2     | 2677; 2932  |
| PleI        | GAGTCNNNN!                | 3     | 889; 1782; 2214   |
| PleI        | !NNNNNGACTC               | 5     | 404; 1304; 1790; 3396; 4273   |
| Ple19I      | CGAT!CG                   | 0     |   |
| PmaCI       | CAC!GTG                   | 0     |   |
| PmeI        | GTTT!AAAC                 | 0     |   |
| PmlI        | CAC!GTG                   | 0     |   |
| PpiI        | GAACNNNNCTCNNNNNNNNNNNN!  | 1     | 1813  |
| PpiI        | !NNNNNNNNGAGNNNNNGTTC     | 1     | 3943  |
| PpiI        | GAGNNNNNGTTCNNNNNNNNNNNN! | 1     | 3975  |
| PpiI        | !NNNNNNNGAACNNNNNNCTC     | 1     | 1781  |
| PpsI        | GAGTCNNNN!                | 3     | 889; 1782; 2214   |
| PpsI        | !NNNNNGACTC               | 5     | 404; 1304; 1790; 3396; 4273   |
| Ppu10I      | A!TGCAT                   | 3     | 2299; 2371; 4702  |
| Ppu21I      | YAC!GTR                   | 3     | 340; 1847; 3035   |
| PpuMI       | RG!GWCCY                  | 0     |   |
| PscI        | A!CATGT                   | 1     | 4649  |
| PshAI       | GACNN!NNGTC               | 1     | 1234  |

| Enzyme name | Sequence                     | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|------------------------------|-------|---|
| PshBI       | AT!TAAT                      | 1     | 7   |
| PsiI        | TTA!TAA                      | 2     | 1514; 1719  |
| Psp03I      | GGWC!C                       | 2     | 3250; 3695  |
| Psp5II      | RG!GWCCY                     | 0     |   |
| Psp6I       | !CCWGG                       | 17    | 88; 281; 776; 824; 920; 935; 1064; 1173; 2265; 2320; 2337; 3116; 3695; 3798; 4488; 4501; 4622   |
| Psp1406I    | AA!CGTT                      | 0     |   |
| Psp124BI    | GAGCT!C                      | 1     | 1326  |
| PspCI       | CAC!GTG                      | 0     |   |
| PspEI       | G!GTNACC                     | 0     |   |
| PspGI       | !CCWGG                       | 17    | 88; 281; 776; 824; 920; 935; 1064; 1173; 2265; 2320; 2337; 3116; 3695; 3798; 4488; 4501; 4622   |
| PspLI       | C!GTACG                      | 0     |   |
| PspN4I      | GGN!NCC                      | 20    | 467; 657; 717; 1108; 1353; 1361; 1368; 1886; 1907; 1919; 2110; 2271; 2343; 2732; 2767; 3615; 3694; 3739; 4582; 4621   |
| PspOMI      | G!GGCCC                      | 1     | 1359  |
| PspOMII     | CGCCARNNNNNNNNNNNNNNNNNNNNN! | 3     | 125; 2461; 3460   |
| PspOMII     | !NNNNNNNNNNNNNNNNNNNYTGGGCG  | 1     | 2863  |
| PspPI       | G!GNCC                       | 12    | 83; 276; 751; 994; 1086; 1360; 1838; 3247; 3692; 3738; 3796; 3829   |
| PspPPI      | RG!GWCCY                     | 0     |   |
| PspPRI      | CCYCAGNNNNNNNNNNNNNNNN!      | 3     | 2284; 2356; 3862  |
| PspPRI      | !NNNNNNNNNNNNNNNNCTGRGG      | 4     | 846; 1274; 2197; 3392   |
| PspXI       | VC!TCGAGB                    | 0     |   |
| PsrI        | GAACNNNNNNNTACNNNNNNNNNNNN!  | 0     |   |
| PsrI        | !NNNNNNNGTANNNNNNGTTC        | 0     |   |
| PsrI        | GTANNNNNNGTTCNNNNNNNNNNNN!   | 0     |   |
| PsrI        | !NNNNNNNGAACNNNNNNNTAC       | 0     |   |
| PssI        | RGGNC!CY                     | 1     | 3832  |
| PstI        | CTGCA!G                      | 1     | 1344  |
| PstNI       | CAGNNN!CTG                   | 3     | 1268; 3832; 4240  |
| PsuI        | R!GATCY                      | 12    | 585; 1141; 1315; 1366; 1375; 2902; 3148; 3540; 3899; 3911; 3997; 4008   |
| PsyI        | GACN!NNGTC                   | 1     | 2849  |
| PteI        | G!CGCGC                      | 0     |   |
| PvuI        | CGAT!CG                      | 0     |   |
| PvuII       | CAG!CTG                      | 2     | 2229; 2837  |
| RcaI        | T!CATGA                      | 2     | 2157; 3929  |
| RceI        | CATCGACNNNNNNNNNNNNNNNNNNNN! | 1     | 3246  |
| RceI        | !NNNNNNNNNNNNNNNNNNNGTCGATG  | 0     |   |
| RgaI        | GCGAT!CGC                    | 0     |   |
| RigI        | GGCCGG!CC                    | 0     |   |
| RleAI       | CCCACANNNNNNNNNNNNNN!        | 1     | 1460  |
| RleAI       | !NNNNNNNNNTGTGGG             | 0     |   |
| RpaBI       | CCCGCAGNNNNNNNNNNNNNNNNNNNN! | 0     |   |
| RpaBI       | !NNNNNNNNNNNNNNNNNNNNCTGCGGG | 0     |   |
| RpaB5I      | CGRGGACNNNNNNNNNNNNNNNNNNNN! | 0     |   |
| RpaB5I      | !NNNNNNNNNNNNNNNNNNNGTCCYCG  | 0     |   |
| RruI        | TCG!CGA                      | 0     |   |
| RsaI        | GT!AC                        | 10    | 219; 244; 299; 332; 383; 540; 646; 1048; 1353; 3037   |
| RsaNI       | G!TAC                        | 10    | 218; 243; 298; 331; 382; 539; 645; 1047; 1352; 3036   |
| RseI        | CAYNN!NNRTG                  | 3     | 365; 634; 3168  |
| RsrII       | CG!GWCCG                     | 1     | 3247  |
| Rsr2I       | CG!GWCCG                     | 1     | 3247  |
| SacI        | GAGCT!C                      | 1     | 1326  |
| SacII       | CCGC!GG                      | 1     | 1358  |
| SalI        | G!TCGAC                      | 1     | 1345  |
| SapI        | GCTCTCN!                     | 0     |   |
| SapI        | !NNNNGAAGAGC                 | 2     | 3075; 3285  |
| SaqAI       | T!TAA                        | 17    | 7; 620; 1301; 1432; 1493; 1614; 1635; 1646; 1658; 1669; 1686; 1784; 2055; 3874; 3888; 3893; 3945  |
| SatI        | GC!NGC                       | 33    | 750; 801; 945; 1062; 1243; 1508; 2027; 2049; 2063; 2501; 2640; 2692; 2703; 2793; 2798; 2835; 2876; 2963; 2966; 2969; 3205; 3301; 3342; 3356; 3470; 3809; 3824; 4035; 4241; 4244; 4309; 4452; 4607 |



| Enzyme name | Sequence                    | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|-----------------------------|-------|--|
| Sau96I      | G!GNCC                      | 12    | 83; 276; 751; 994; 1086; 1360; 1838; 3247; 3692; 3738; 3796; 3829  |
| Sau3AI      | !GATC                       | 24    | 585; 965; 1115; 1141; 1315; 1366; 1375; 1388; 2568; 2572; 2591; 2902; 2980; 3061; 3070; 3148; 3524; 3540; 3899; 3911; 3989; 3997; 4008; 4083   |
| SbfI        | CCTGCA!GG                   | 0     |  |
| ScaI        | AGT!ACT                     | 0     |  |
| SchI        | GAGTCNNNNN!                 | 3     | 890; 1783; 2215  |
| SchI        | !NNNNNGACTC                 | 5     | 404; 1304; 1790; 3396; 4273  |
| SciI        | CTC!GAG                     | 1     | 1321   |
| ScrFI       | CC!NGG                      | 23    | 90; 283; 778; 826; 922; 937; 1066; 1175; 1363; 1364; 2267; 2322; 2339; 2735; 2895; 3118; 3508; 3697; 3800; 4272; 4490; 4503; 4624  |
| SdaI        | CCTGCA!GG                   | 0     |  |
| SdeAI       | CAGRAGNNNNNNNNNNNNNNNNNNNN! | 5     | 1032; 2309; 2381; 2550; 4492   |
| SdeAI       | !NNNNNNNNNNNNNNNNNNNNCTYCTG | 2     | 2886; 3371   |
| SdeOSI      | GACNNNNRTGANNNNNNNNNNN!     | 1     | 279  |
| SdeOSI      | !NNNNNNNNNNNTCAYNNNNNGTC    | 0     |  |
| SdeOSI      | TCAYNNNNNGTCNNNNNNNNNNNN!   | 0     |  |
| SdeOSI      | !NNNNNNNNNNNGACNNNNRTGA     | 1     | 245  |
| SduI        | GDGCH!C                     | 14    | 658; 685; 718; 1295; 1326; 1363; 1922; 2677; 2770; 2844; 3034; 3096; 3806; 4339  |
| SelI        | !CGCG                       | 16    | 58; 1355; 1617; 1653; 2029; 2049; 2073; 2104; 2795; 3096; 3534; 3618; 3681; 3750; 4023; 4604   |
| SetI        | ASST!                       | 64    | 125; 137; 178; 261; 308; 342; 447; 552; 568; 617; 644; 740; 790; 811; 962; 1097; 1166; 1234; 1238; 1253; 1277; 1301; 1326; 1332; 1421; 1442; 1451; 1463; 1512; 1679; 1794; 1806; 1849; 1886; 1936; 1959; 2083; 2231; 2326; 2519; 2632; 2760; 2839; 2850; 2922; 2993; 3037; 3297; 3443; 3487; 3818; 3849; 3908; 3951; 4094; 4115; 4214; 4351; 4366; 4385; 4397; 4441; 4487; 4538  |
| SexAI       | A!CCWGGT                    | 1     | 2320   |
| SfaAI       | GCGAT!CGC                   | 0     |  |
| SfaNI       | GCATCNNNNN!                 | 7     | 839; 1547; 2310; 2382; 3030; 3094; 3369  |
| SfaNI       | !NNNNNNNNNGATGC             | 7     | 357; 992; 1160; 2689; 2944; 3160; 4552   |
| SfcI        | C!TRYAG                     | 5     | 1194; 1340; 2066; 4193; 4384   |
| SfiI        | GGCCNNNN!NGGCC              | 1     | 2506   |
| SfoI        | GGC!GCC                     | 1     | 2732   |
| Sfr274I     | C!TCGAG                     | 1     | 1319   |
| Sfr303I     | CCGC!GG                     | 1     | 1358   |
| SfuI        | TT!CGAA                     | 2     | 1333; 3413   |
| SgeI        | CNNGNNNNNNNNN!              | 188   | 6; 36; 71; 97; 123; 223; 236; 252; 290; 307; 336; 373; 407; 426; 491; 548; 579; 604; 618; 642; 660; 690; 714; 732; 747; 789; 816; 837; 858; 871; 884; 912; 933; 948; 975; 1009; 1030; 1050; 1063; 1077; 1095; 1108; 1140; 1158; 1179; 1213; 1239; 1258; 1274; 1292; 1308; 1331; 1368; 1385; 1438; 1467; 1509; 1592; 1630; 1666; 1750; 1772; 1804; 1841; 1859; 1878; 1893; 1935; 1952; 1969; 2004; 2018; 2035; 2057; 2081; 2105; 2170; 2184; 2222; 2238; 2278; 2291; 2310; 2333; 2350; 2363; 2382; 2412; 2433; 2450; 2473; 2508; 2535; 2566; 2588; 2614; 2636; 2655; 2683; 2719; 2738; 2761; 2775; 2795; 2808; 2822; 2841; 2855; 2874; 2906; 2920; 2933; 2959; 2990; 3003; 3020; 3037; 3051; 3068; 3086; 3101; 3129; 3145; 3164; 3186; 3202; 3225; 3244; 3269; 3288; 3308; 3335; 3359; 3392; 3411; 3439; 3453; 3519; 3543; 3558; 3581; 3602; 3619; 3667; 3694; 3708; 3737; 3761; 3794; 3811; 3831; 3844; 3857; 3915; 3942; 3961; 3991; 4016; 4036; 4050; 4075; 4093; 4130; 4168; 4197; 4225; 4239; 4259; 4274; 4295; 4309; 4328; 4346; 4362; 4402; 4422; 4449; 4467; 4488; 4501; 4514; 4531; 4555; 4578; 4610; 4635; 4662; 4684 |

| Enzyme name | Sequence                       | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|--------------------------------|-------|--|
| SgeI        | !NNNNNNNNNNNNNCNNG             | 166   | 10; 45; 71; 97; 197; 226; 264; 281; 310; 347; 381; 400; 465; 522; 553; 578; 598; 616; 634; 664; 688; 706; 730; 763; 790; 811; 832; 858; 886; 907; 949; 983; 1004; 1024; 1051; 1069; 1090; 1114; 1132; 1153; 1187; 1213; 1232; 1266; 1293; 1313; 1342; 1359; 1412; 1441; 1483; 1566; 1604; 1640; 1724; 1746; 1778; 1815; 1833; 1852; 1909; 1926; 1943; 1978; 1996; 2016; 2036; 2055; 2079; 2144; 2196; 2252; 2284; 2307; 2324; 2356; 2386; 2407; 2424; 2447; 2482; 2509; 2540; 2562; 2588; 2610; 2629; 2657; 2693; 2712; 2735; 2756; 2774; 2791; 2815; 2848; 2880; 2907; 2933; 2964; 2994; 3011; 3037; 3060; 3081; 3103; 3123; 3143; 3160; 3199; 3218; 3243; 3262; 3282; 3309; 3333; 3366; 3385; 3413; 3435; 3493; 3517; 3536; 3555; 3576; 3593; 3641; 3668; 3688; 3711; 3735; 3768; 3785; 3805; 3831; 3889; 3916; 3935; 3965; 3990; 4010; 4049; 4067; 4104; 4142; 4171; 4199; 4220; 4248; 4269; 4302; 4320; 4376; 4396; 4423; 4441; 4462; 4488; 4505; 4529; 4552; 4584; 4609; 4636; 4658; 4687 |
| SgfI        | GCGAT!CGC                      | 0     |  |
| SgrAI       | CR!CCGGYG                      | 0     |  |
| SgrBI       | CCGC!GG                        | 1     | 1358   |
| SgrDI       | CG!TCGACG                      | 0     |  |
| SgsI        | GG!CGCGCC                      | 0     |  |
| SimI        | GG!GTC                         | 4     | 27; 1878; 3665; 4458   |
| SimI        | !GACCC                         | 4     | 106; 3159; 3727; 3975  |
| SinI        | G!GWCC                         | 2     | 3247; 3692   |
| SlaI        | C!TCGAG                        | 1     | 1319   |
| SmaI        | CCC!GGG                        | 1     | 1364   |
| SmiI        | ATTT!AAAT                      | 0     |  |
| SmiMI       | CAYNN!NNRTG                    | 3     | 365; 634; 3168   |
| SmlI        | C!TYRAG                        | 7     | 1165; 1319; 1325; 3122; 4004; 4281; 4543   |
| SmoI        | C!TYRAG                        | 7     | 1165; 1319; 1325; 3122; 4004; 4281; 4543   |
| SmuI        | CCCGCNNNN!                     | 10    | 93; 119; 286; 1059; 1161; 2053; 2408; 2429; 3625; 3757   |
| SmuI        | !NNNNNNGCGGG                   | 7     | 1096; 1350; 1984; 2854; 3391; 3529; 3676   |
| SnaBI       | TAC!GTA                        | 1     | 340  |
| SpeI        | A!CTAGT                        | 0     |  |
| SphI        | GCATG!C                        | 3     | 2301; 2373; 3136   |
| SrfI        | GCCC!GGGC                      | 0     |  |
| Sse9I       | !AATT                          | 12    | 18; 1302; 1335; 1481; 1545; 1623; 1649; 1660; 2307; 2379; 2471; 3889   |
| Sse8387I    | CCTGCA!GG                      | 0     |  |
| Sse8647I    | AG!GWCCCT                      | 0     |  |
| SseBI       | AGG!CCT                        | 2     | 1030; 2552   |
| SsiI        | C!CGC                          | 35    | 58; 86; 98; 112; 279; 507; 589; 914; 1052; 1154; 1355; 2046; 2049; 2063; 2153; 2401; 2413; 2422; 2434; 2444; 2455; 2501; 2640; 2703; 3205; 3250; 3316; 3342; 3467; 3470; 3618; 3750; 4057; 4201; 4697  |
| SsiI        | G!CGG                          | 28    | 370; 403; 528; 595; 749; 1061; 1103; 1357; 1991; 2018; 2106; 2216; 2797; 2861; 2962; 2965; 3245; 3300; 3398; 3536; 3683; 3823; 4066; 4311; 4432; 4451; 4578; 4606  |
| SspI        | AAT!ATT                        | 2     | 1639; 2192   |
| SspDI       | G!GCGCC                        | 1     | 2730   |
| SspD5I      | GGTGANNNNNNNN!                 | 4     | 376; 987; 3802; 3918   |
| SspD5I      | !NNNNNNNNNTCACC                | 4     | 875; 1025; 1847; 2908  |
| SstI        | GAGCT!C                        | 1     | 1326   |
| SstII       | CCGC!GG                        | 1     | 1358   |
| SstE37I     | CGAAGACNNNNNNNNNNNNNNNNNNNNNN! | 1     | 920  |
| SstE37I     | !NNNNNNNNNNNNNNNNNNNGTCTTCG    | 0     |  |
| Sth132I     | CCCGNNNN!                      | 19    | 92; 118; 285; 842; 1058; 1160; 1369; 1930; 2052; 2407; 2428; 2741; 3144; 3283; 3354; 3624; 3756; 3986; 4424  |
| Sth132I     | !NNNNNNNNNCGGG                 | 21    | 15; 407; 470; 891; 1095; 1349; 1931; 1983; 2084; 2811; 2853; 2886; 3390; 3499; 3528; 3675; 3779; 3810; 4263; 4307; 4515  |
| Sth302II    | CC!GG                          | 19    | 1308; 1364; 1374; 1950; 2637; 2714; 2736; 2764; 2895; 2985; 3052; 3233; 3508; 3516; 3608; 4082; 4272; 4298; 4445   |
| StrI        | C!TCGAG                        | 1     | 1319   |
| StsI        | GGATGNNNNNNNNNN!               | 4     | 2599; 3056; 3081; 3535   |
| StsI        | !NNNNNNNNNNNNNNCATCC           | 5     | 671; 759; 816; 971; 2403   |
| StuI        | AGG!CCT                        | 2     | 1030; 2552   |
| StyI        | C!CWWGG                        | 5     | 360; 2460; 2553; 3163; 3568  |

| Enzyme name | Sequence                 | Count | Cutting Position (the base to the left of the cut site)   |
|-------------|--------------------------|-------|---|
| StyD4I      | !CCNGG                   | 23    | 88; 281; 776; 824; 920; 935; 1064; 1173; 1361; 1362; 2265; 2320; 2337; 2733; 2893; 3116; 3506; 3695; 3798; 4270; 4488; 4501; 4622 |
| SwaI        | ATTT!AAAT                | 0     |   |
| TaaI        | ACN!GT                   | 15    | 76; 198; 269; 543; 581; 660; 1351; 1822; 3111; 3227; 3335; 3658; 4149; 4619; 4690   |
| TaiI        | ACGT!                    | 16    | 125; 137; 178; 261; 342; 447; 962; 1238; 1794; 1806; 1849; 1959; 2850; 3037; 3818; 3951   |
| TaqI        | T!CGA                    | 17    | 743; 770; 1238; 1320; 1333; 1346; 1880; 2571; 2844; 3000; 3024; 3060; 3222; 3413; 3458; 3713; 4551                                |
| TaqII       | GACCGANNNNNNNNNN!        | 3     | 1751; 2768; 3436  |
| TaqII       | !NNNNNNNNNTCGGTC         | 1     | 3680  |
| TaqII       | CACCCANNNNNNNNNN!        | 2     | 733; 838  |
| TaqII       | !NNNNNNNNNTGGGTG         | 3     | 172; 2635; 3227   |
| TasI        | !AATT                    | 12    | 18; 1302; 1335; 1481; 1545; 1623; 1649; 1660; 2307; 2379; 2471; 3889  |
| TatI        | W!GTACW                  | 5     | 217; 297; 330; 381; 644   |
| TauI        | GCSG!C                   | 17    | 752; 1064; 2051; 2065; 2503; 2642; 2705; 2800; 2965; 2968; 3207; 3303; 3344; 3472; 3826; 4454; 4609                               |
| TfiI        | G!AWTC                   | 7     | 731; 1208; 3216; 3350; 3460; 3497; 4675   |
| TliI        | C!TCGAG                  | 1     | 1319  |
| TruII       | T!TAA                    | 17    | 7; 620; 1301; 1432; 1493; 1614; 1635; 1646; 1658; 1669; 1686; 1784; 2055; 3874; 3888; 3893; 3945                                  |
| Tru9I       | T!TAA                    | 17    | 7; 620; 1301; 1432; 1493; 1614; 1635; 1646; 1658; 1669; 1686; 1784; 2055; 3874; 3888; 3893; 3945                                  |
| TscAI       | CASTGNN!                 | 6     | 1262; 1577; 2860; 3970; 4241; 4254  |
| TseI        | G!CWGC                   | 16    | 800; 944; 1242; 1507; 2026; 2691; 2792; 2834; 2875; 2968; 3355; 3808; 4034; 4240; 4243; 4308                                      |
| TsoI        | TARCCANNNNNNNNNN!        | 2     | 1703; 2050  |
| TsoI        | !NNNNNNNNNTGGYTA         | 3     | 769; 1508; 3260   |
| Tsp45I      | !GTSAC                   | 4     | 882; 2020; 2851; 3157   |
| Tsp509I     | !AATT                    | 12    | 18; 1302; 1335; 1481; 1545; 1623; 1649; 1660; 2307; 2379; 2471; 3889  |
| TspDTI      | ATGAANNNNNNNNNN!         | 4     | 652; 1489; 2790; 3494   |
| TspDTI      | !NNNNNNNNNTTCAT          | 5     | 26; 801; 3208; 3664; 3871   |
| TspEI       | !AATT                    | 12    | 18; 1302; 1335; 1481; 1545; 1623; 1649; 1660; 2307; 2379; 2471; 3889  |
| TspGWI      | ACGGANNNNNNNNNN!         | 1     | 3606  |
| TspGWI      | !NNNNNNNNNTCCGT          | 0     |   |
| TspMI       | C!CCGGG                  | 1     | 1362  |
| TspRI       | CASTGNN!                 | 6     | 1262; 1577; 2860; 3970; 4241; 4254  |
| TstI        | CACNNNNNTCCNNNNNNNNNN!   | 0     |   |
| TstI        | !NNNNNNNGGANNNNNNGTG     | 0     |   |
| TstI        | GGANNNNNGTGNNNNNNNNNNNN! | 0     |   |
| TstI        | !NNNNNNNCCACNNNNNTCC     | 0     |   |
| Tth111I     | GACN!NNGTC               | 1     | 2849  |
| Tth111II    | CAARCAANNNNNNNNNN!       | 3     | 862; 1137; 4058   |
| Tth111III   | !NNNNNNNNNTGYTTG         | 3     | 3166; 4026; 4065  |
| UnbI        | !GGNCC                   | 12    | 82; 275; 750; 993; 1085; 1359; 1837; 3246; 3691; 3737; 3795; 3828   |
| Van91I      | CCANNNN!NTGG             | 0     |   |
| Vha464I     | C!TTAAG                  | 0     |   |
| VneI        | G!TGCAC                  | 2     | 681; 4335   |
| VpaK11AI    | !GGWCC                   | 2     | 3246; 3691  |
| VpaK11BI    | G!GWCC                   | 2     | 3247; 3692  |
| VspI        | AT!TAAT                  | 1     | 7   |
| XagI        | CCTNN!NNNAGG             | 0     |   |
| XapI        | R!AATTY                  | 4     | 1335; 1545; 1649; 1660  |
| XbaI        | T!CTAGA                  | 1     | 1378  |
| XceI        | RCATG!Y                  | 5     | 633; 2301; 2373; 3136; 4653   |
| XcmI        | CCANNNN!NNNTGG           | 0     |   |
| XhoI        | C!TCGAG                  | 1     | 1319  |
| XhoII       | R!GATCY                  | 12    | 585; 1141; 1315; 1366; 1375; 2902; 3148; 3540; 3899; 3911; 3997; 4008   |
| XmaI        | C!CCGGG                  | 1     | 1362  |
| XmaJI       | C!CTAGG                  | 2     | 2553; 3568  |

| Enzyme name | Sequence    | Count | Cutting Position (the base to the left of the cut site)  |
|-------------|-------------|-------|--|
| XmiI        | GT!MKAC     | 2     | 1181; 1346   |
| XmnI        | GAANN!NNTTC | 0     |  |
| XspI        | C!TAG       | 10    | 592; 785; 1280; 1379; 1580; 1998; 2554; 3569; 3903; 4156 |
| ZraI        | GAC!GTC     | 4     | 123; 176; 259; 445                                       |
| ZrmI        | AGT!ACT     | 0     |  |
| Zsp2I       | ATGCA!T     | 3     | 2303; 2375; 4706   |